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[1] As of January 1, 1960.
[2] On leave to pursue studies.
[3] On other types of leave.
[4] U. S. Department of Agriculture collaborators.

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A Modification of the *Lemna* Test for Phytotoxicity

Fritz Fromm[1, 2]

INTRODUCTION

Chemical weed controls are employed more and more in the modernization of agriculture. The controls are of increased importance in the economy of an intensively used land area, such as the farming lands of Puerto Rico. Good control of weeds has to be based on a study of the phytotoxic properties of the compounds applied. Field tests alone cannot be used for this purpose because too many extraneous variables, such as the nature of the soil and the variations of climatic conditions, obscure the results. Hence, a number of laboratory methods have been introduced (see *8*, *16* for reviews[3].) Most of these methods are derived from tests on growth-promoting substances; hence, they can be used for this type of weedkiller only. The *Lemna* test, however, is applicable to weedkillers of all types and appears to be the method of choice for studies of relative phytotoxicity.

Since Hessenland, Fromm, and Saalmann (*14*) first used *Lemna minor* Linn. for comparative studies of phytotoxicity, the method has been altered repeatedly (*3*, *9* to *15*). Most of the modifications suggested were based on the fundamental studies of the physiology of *Lemna* by Ashby, Clark, and their coworkers (*1*, *2*, *5*, *6*, *7*). Work carried out with the idea of developing a more satisfactory *Lemna* test is reported here. As the purpose of the test is the evaluation of the toxic action of chemical compounds, but not the production of optimal growth of the plant, the procedure of Ashby and coworkers (*1*, *2*) was not followed strictly by any of the users of *Lemna* as a test plant.

[1] Biochemist, Department of Plant Pathology and Botany, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R. Part of this work was done in the Department of Botany and Plant Pathology, Ohio State University, and part in the Department of Plant Pathology and Botany, Agricultural Experiment Station, University of P. R.

[2] The author is very much indebted to Chairman B. S. Meyer, Department of Botany and Plant Pathology, Ohio State University, Columbus, Ohio, for his permission to use the facilities of the department for part of the experiments, and to Julio Bird Piñero and Moisés Padilla at this Experiment Station for taking the picture shown as figure 1.

[3] Italic numbers in parentheses refer to Literature Cited, p. 101–2.

PROCEDURES

In recent experiments four 400-ml. beakers, made of resistant laboratory glass, were filled with the pure nutrient [Clark and Roller (*7*)], or the nutrient containing the toxic agent in a given concentration. Twenty plants of *Lemna minor* with two fronds each were planted in each beaker. The plants were all taken from the same strain. In contrast to earlier work done at Pittsburgh, Pa., (*10, 11, 12*) and Santurce, P.R., (*9*) for the experiments reported here, strain 2803, now considered the standard clone, was used. It consists exclusively of descendants of one plant, the cultivation of which was started at Columbus, Ohio, on September 11, 1958. The beakers were covered with petri dishes to avoid concentration of the solutions by evaporation of water, and kept at a window sill or in the greenhouse under natural light, and the number of plants and fronds was determined daily. For the control and the sublethal concentrations of a poison the growth rate K was calculated from the equation $\log (N/N_0) = Kt$ according to Clark (*5*) and the percentage growth inhibition established as $100\ (1 - K/K_0)$, K_0 being the growth rate of the control, N_0 the number of fronds at the start of the experiment, and N the number of fronds after t days.

The growth rate K of the controls varied from 0.028 to 0.051 with a standard error of σ 0.001–0.003, *i.e.*, the results are useful qualitatively rather than quantitatively. The various factors which influence the growth of *Lemna minor* were, therefore, examined in the hope that a better control of these variables would yield more accurate values.

GROWTH-RATE STUDIES

A more constant growth rate of the controls than that attained in the laboratory at Pittsburgh could be achieved by more uniform illumination. In the greenhouse at Columbus, Ohio, controls grew generally with K = 0.05–0.06, under artificial light much higher rates, *e.g.*, 0.11 to 0.12 at 500 foot-candles for 24 hours a day were obtained for the growth of fronds.

Even small differences in illumination produced noticeable differences in the growth rate. In experiments in which the four beakers were arranged vertically with respect to the light source, a definite trend for K was observed, while an arrangement strictly parallel to the windows or light source showed random distribution of K (table 1). Table 1 illustrates the growth rate during a hot summer. Illumination immediately at the window was too strong for optimal growth and the growth rate increased, therefore, with the distance from the window. In the winter months the change in growth rate was reversed for the arrangement vertical to the window; the best conditions of illumination existed directly at the window and the growth rate decreased with the distance from the window.

The growth rate of the plants was not strictly parallel to the increase in the number of fronds and showed a higher standard deviation. Ashby and coworkers (*1*) have already observed a definite rhythm in the growth of *Lemna* plants, but an accurate count of the number of plants appears difficult because during long photoperiods and under continous illumination, *Lemna* tends to form clusters of plants which are difficult to count. Plants with four fronds which are occasionally mentioned in the literature (*1*) are an aggregation of two plants with two fronds each, as can easily be seen by the existence of two roots, and the fact that they separate readily on a light touch or by simple transfer from one solution to another. Even plants with three fronds frequently already have two roots, but they are generally not yet ready to separate.

Figure 1 shows typical forms of the plants. Only the plants with two

TABLE 1.—*Growth rates of fronds of* **Lemna minor** *in Clark's nutrient when beakers containing them were differently arranged*

Vertical to window of greenhouse		Parallel to window of greenhouse	
Position of beaker	$K \pm \sigma$	Beaker No.	$K \pm \sigma$
Nearest to window	0.023 ± 0.003	1	0.069 ± 0.001
1 beaker removed from window	.034 ± .002	2	.070 ± .001
2 beakers removed from window	.033 ± .002	3	.050 ± .002
3 beakers removed from window	.048 ± .002	4	.063 ± .001

fronds at the top right and in the center of the picture are single individuals. All other forms are associations of two plants, as can be seen by the thin dividing lines and, in some cases, by the fact that more than one root is visible. Similar observations on other *Lemnaceae* have also been reported by Yoshimura (*17*). One effect of a toxic agent is also the splitting of the plants into individuals with one frond each, which leads to a remarkable increase in the number of plants, but does not indicate any growth, as the fronds begin to die at the same time (*11*, *15*). The evaluation of the growth of *Lemna* was therefore based on the fronds only.

MINIMUM VOLUME OF NUTRIENT

However, the growth rate of the fronds was not constant during the first 1 to 3 days of the experiment. It was suspected that this deviation was due to the relatively large percentage error which 1 frond more or less represents for 40 fronds. A series of experiments in which 80 fronds were used in petri dishes showed no improvement. The growth rate during the first 2 to 3 days differed greatly from that observed during the following

5 or 6 days. An unexpected result was a significantly lower growth rate in the experiments with 80 plants in petri dishes (60-ml. capacity) than in controls run with 40 plants in 400-ml. beakers (table 2).

The conclusion that the lowering of K was caused by starvation, *i.e.*, an insufficient volume of nutrient, was confirmed in experiments like the second series in table 2, in which the *Lemna* was started on 100 ml. of nutrient, and was transferred to fresh nutrient every day. Under these conditions

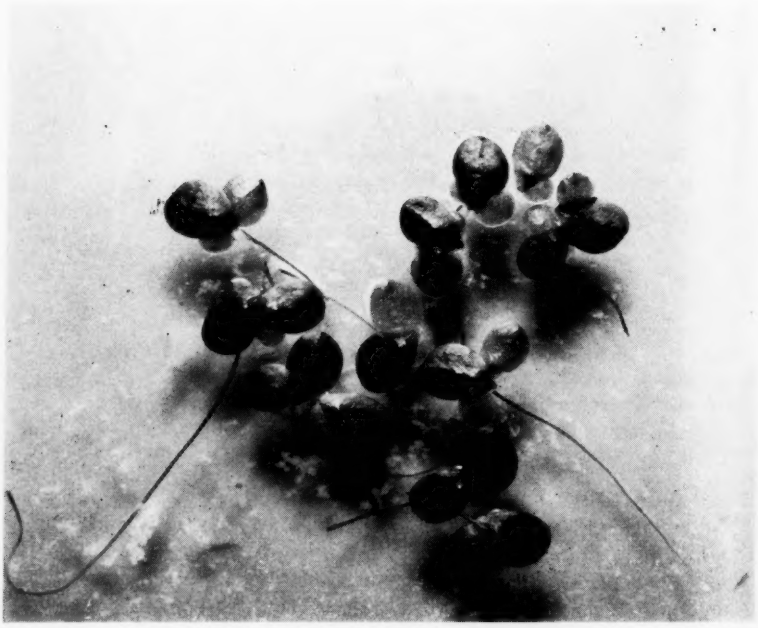

FIG. 1.—Typical plants and clusters of *Lemna minor* enlarged about 2×.

growth in the smaller volume was not significantly different from that of the control. Eventually, a volume of 1 to 2 ml. of nutrient per plant with two fronds per day was established as the minimum requirement for good growth.

Hence, it was concluded that the total volume used must be adjusted to the population size, *i.e.*, the experiment with 20 plants per beaker could be started in 50-ml. beakers, but a larger beaker should be substituted as soon as >50 plants, meaning >100 fronds, have appeared.

Frequent changes of the nutrient solution have already been recommended by Clark (*5*). He introduced the numerous changes to avoid the cumbersome aeration of the solutions and to control the growth of algae

in them. These are not the only advantages obtained by a daily change of solution. It is to be expected that the growth of any plant on a given volume of nutrient will decrease the concentration of the dissolved salts continuously. This decrease can easily be followed by conductivity measurements. Typical data on this change and its effect are shown in table 3. The growth of 20 plants with 2 fronds in each of four 50-ml. beakers, in which the nutrient was changed daily, was compared with that of the same number of plants in four 250-ml. beakers in which the solution was not changed during the 7 days of the experiment. The resistance of the solutions was measured every day.

Generally, the growth rate in the unchanged solution was not significantly different from that of the plants in solutions which were changed daily, but occasionally a significant decrease in growth rate (of about 10 percent) was seen in the unchanged nutrient. More important is the fact that frequently the growth rate in the unchanged nutrient is not constant

TABLE 2.—*Results of growth experiments with* **Lemna minor** *in different volumes of Clark's nutrient*

Volume of nutrient (ml. daily)	Original number of fronds	$K \pm \sigma$	$(100K)/K_0$	T
400	40	0.076 ± 0.004	100	—
60	80	.035 ± .005	46	6.19
400	40	.050 ± .003	100	—
100	40	.048 ± .002	95	.76
60	40	.033 ± .006	66	2.47

at all, but decreases steadily from day to day, *e.g.*, while K for the experiment, with a daily change of nutrient was 0.1091 ± 0.0002, it diminished continuously in the unchanged nutrient from 0.1188 on the third day to 0.1024 on the ninth day.

Similar concentration changes are to be expected for the toxic agent dissolved in the nutrient. Direct evidence is difficult to obtain as most of the toxicants show very little conductance (if any) and their initial concentration is already low.[4]

[4] After completion of this manuscript the paper: The Uptake of Growth Substances, I: Factors controlling the uptake of phenoxyacetic acids by *Lemna minor*, by G. E. Blackman, G. Sen, W. R. Birch, and R. G. Powell, *J. Exp. Bot.* **10**, 33–54 (1959), came to my attention, *C. A.* **54**, 2504 *f* (1960). Their statement that 23 to 25 ml. of nutrient are satisfactory for 115 to 120 fronds for 4 hours is in good agreement with my estimate of 1 to 2 ml./day for 2 fronds. The simultaneous uptake and loss of toxicant by the plant which they describe is a further argument for a daily change of the experimental solution, as a decreasing concentration of this solution would create a concentration gradient, the influence of which on the toxicant concentration in the plant would be difficult to determine.

EFFECTS OF PREVIOUS HISTORY OF PLANT MATERIAL

The daily change to a fresh quantity of at least the minimum volume of nutrient, however, did not explain or resolve the problem of the irregular growth at the start of the experiment. Ashby and Oxley (*2*) have already pointed out that the previous history of the plants influences the outcome of the experiment, but they did not give any data on the intensity or duration of its influence on the growth of the plant.

One of our cultures which suffered a severe Mn deficiency and had a growth rate K of 0.029 when exposed to 400 foot-candles needed 6 days after the addition of the Mn to reach the new constant growth rate of 0.071 ± 0.001. However, in subsequent runs it was proved that the recovery was not yet complete; for the growth rate in individual beakers receiving identical treatments continued to show fluctuations of $\pm$ 20

TABLE 3.—*Effects on growth of* **Lemna minor** *of a change of the nutrient and its concentration during 7 days*

Volume of nutrient (ml.) and changes	$K \pm \sigma$	$100K/K_0$	T	Conductance in mho $\times 10^{-5}$		
				0 day	2nd day	Last day
50, daily	0.111 ± 0.003	100	—	122	122	122
250, none	$.115 \pm .002$	104	1.27	122	118	104
50, daily	$.123 \pm .001$	100	—	118	118	118
250, none	$.126 \pm .001$	103	1.86	118	105	98

percent with each other. Only after about 5 weeks of uniform cultivation did parallel beakers give constant growth rates with a standard error of $\pm$ 0.003. Similarly, in an experiment in which survivors of poisoning with 0.01 M ethylamine were transferred after 13 days to pure nutrient, the growth rate showed an immediate increase and, after 4 days, was better than that of the control, but the plants and fronds were still exceedingly small when the run had to be terminated on the 19th day of the experiment. Similar periods of adaption were observed when the intensity of illumination was changed.

Even much smaller changes in previous history of test plants produced significant changes in the growth rate. For example, in one series four beakers were planted with stock of clone 2803 which had been growing well, but had then been kept on the same nutrient solution for 22 days, while another four beakers were planted with *Lemna* of standard clone 2803, the nutrient of which had been changed daily. Both sets of beakers were kept in the greenhouse under identical conditions with daily renewal of the nutrient for 11 days. The growth rate of the second set of beakers

was 0.0603 ± 0.0004, while the fronds in the first set grew at $K = 0.051 \pm 0.001$, *i.e.*, 85 percent *as well* (T value 11.04) as that of the second set. In addition, the standard error was more than twice as high, indicating that the growth rates in the individual beakers of the first set fluctuated much more than those of the second set. In another case plants with two fronds each were growing with a value of $K = 0.120 \pm 0.001$. These were being illuminated at 500 foot-candles for 24 hours a day, the solution had pH 6.8 at a temperature of 23° ± 1°. These plants were grown on nutrient which was changed daily. After transplanting these plants to a new series under the same conditions the growth value was found to be $K = 0.123 \pm 0.001$. However, the count on the first day of the new run continued to be irregular.

Therefore, in an attempt to overcome growth irregularity, the plants were passed through a preliminary cultivation under conditions identical with the control of the experiment planned and used only after they had shown a constant growth rate for from 5 to 7 days. Under these conditions the controls would reach a constant growth rate on the second day of the experiment. If the stock of plants was kept on the same, and frequently changed nutrient, as the control, it was found that a preparatory run of about 1 week was sufficient. Often, the controls of the previous experiment can be used directly as material for the next test.

In the course of these studies widely varying growth rates of *Lemna*, clone 2803, have been used. Hence, the question whether toxicity assays at these different K values are comparable, had to be considered. Blackman and Robertson-Cuninghame (*4*) have shown that the response of *Lemna minor* to 2,4-D increased with increasing temperature, and that a light intensity of 700 foot-candles produced a slightly stronger growth inhibition by 2,4-D than lower intensities. Our data, especially on the action of 10^{-2} M ethylamine and 10^{-3} M aniline at growth rates from 0.015 to 0.110, showed that $K < 0.02$ is liable to give irregular results while at $K > 0.06$ the response to killing concentrations may take several days longer than at $0.02 < K < 0.06$. The different growth rates in these experiments were produced by partial starvation and (or) variations in the temperature from 17 to 27° C.; the illumination was always 500 foot-candles. As the purpose of the test is the determination of the *relative* toxicity, work under as nearly as possible constant conditions is a requisite. For practical reasons, a temperature of 24 ± 1° C. and an illumination of 400 foot-candles is preferred.

THE MODIFIED PROCEDURE

The results and observations described above were incorporated in our present testing process in the following manner:

Ten milliliters of solution containing 10 gm. of $CaHPO_4$ per liter, 8 ml. of *M* KNO_3, 1 ml. of *M* $MgSO_4$, 3 drops of *M* $FeSO_4$, and 4 drops 0.1*M* $MnSO_4$ or $MnCl_2$ were filled up to 1 l. with water which was distilled twice in all-glass equipment. Commercially available c.p. chemicals were used for the stock solutions. Eighty *Lemna minor* plants from clone 2803, each with two fronds, were distributed in four covered 50-ml. beakers containing this nutrient, placed parallel to the light source, and grown at the light intensity, (*e.g.* 400 foot-candles), pH (*e.g.* 6.5), and temperature (*e.g.* 24° C.) planned for the following test. Daily changes of the nutrient and counts of the fronds were continued until *K* was reasonably constant for at least 5 days.

Then four 50-ml. beakers each for nutrient and each of the concentrations of the toxicant were prepared and each was seeded with 20 plants, 40 fronds (N_0)—only 10 plants are used if the experiment is to be continued for more than 8 days—of the *Lemna* from the prepared test plants. Plants with two roots or a sharp dividing line between fronds were excluded. No clusters of plants were transplanted. If the number of single plants with two fronds was not sufficient for the start of the experiment, the clusters were separated into their components and the suitable individuals used. Daily, all solutions were changed and the number *N* of fronds was determined at intervals of 24 $\pm$ 1 hour, generally at the time of transfer to fresh solution. The value *K* for each beaker and day was calculated as log (N/N_0)/number of days. From these daily values the mean growth rate K_b per beaker was calculated. From the four K_b of each set (including the control) the average *K* for each solution was found.

SUMMARY

1. The count of fronds of *Lemna minor* is more suitable for the determination of the growth rate than that of the number of plants.

2. The minimum volume of Clark's nutrient solution for one plant with two fronds is 1 ml. per day.

3. The growth of *Lemna minor* in nutrient solution produces measurable changes in nutrient concentration in 24 hours. A daily change of the solution is, therefore, advisable. This applies also to the nutrient containing the toxic agent, as a decrease in concentration is likely to occur there as well.

4. The previous history of the plants manifests itself in an irregular growth rate during the first days of the experiment, if their history is different from the conditions of the experiment. Plants to be used for a test should, therefore, be cultivated under the conditions to be applied for the control until their growth rate is reasonably constant.

5. The modified method for the *Lemna* test for phytotoxicity is described.

RESUMEN

1. El contaje de las frondas de *Lemna minor* se presta más para determinar el promedio de crecimiento que el número de plantas.

2. El volumen mínimo de la solución nutritiva de Clark para una planta con dos frondas es de 1 ml. diario.

3. El crecimiento de *Lemna minor* en solución de nutrientes produce cambios mensurables en la concentración de nutrientes en un período de 24 horas. Un cambio diario de la solución es, en consecuencia, aconsejable. Esto también se aplica al nutriente que contiene el agente tóxico, así como es posible que ocurra una reducción en la concentración.

4. La historia previa de las plantas se evidencia en un promedio de crecimiento irregular durante los primeros días del experimento, siempre que la historia sea diferente de las condiciones del experimento. Las plantas que van a usarse en una prueba deben cultivarse bajo condiciones que vayan a aplicarse para el control, hasta que el promedio de crecimiento de ellas sea razonablemente constante.

5. Se describe aquí el método modificado para la prueba de fitotoxicidad con *Lemna*.

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Digestibility Studies on Venezuela Grass (*Paspalum fasciculatum*) and Plantain Pseudostalks (*Musa paradisiaca*)

J. A. Arroyo and L. Rivera Brenes[1]

INTRODUCTION

The data reported in this paper are the results of digestion trials performed on Venezuela grass (*Paspalum fasciulatum*) and plantain pseudostalks (*Musa paradisiaca*). Digestion trials and palatability tests were conducted concurrently with these two forages. Palatability results were reported separately (*4*).[2]

Venezuela grass was introduced into Puerto Rico from Venezuela some years ago. It is known there as "chiguirera" grass (*3*). Observations made at this Station indicated that it was not well accepted by cattle (*5*). This was later confirmed when it was compared to Merker grass (a strain of Napier grass) in several palatability trials (*4*).

Plantain pseudostalks are sometimes used as forage for cattle here in Puerto Rico and in Central and South America. They are very palatable to cattle (*4*). However, the stalks are very high in moisture content and are recommended only as emergency feed during prolonged dry periods when other roughages are very scarce.

In Puerto Rico thousands of tons of plantain and banana pseudostalks are thrown away during the harvest of these fruits. Since plantain stalks seem to be so palatable to dairy cows it was considered advisable to determine their feeding value.

This study reports the work performed to evaluate the feeding value of plantain pseudostalks and Venezuela grass. The digestible protein and digestible energy were the criteria used, as recommended by Swift (*6*).

PROCEDURE

The experiment consisted of one trial of each of the two roughages with a 7-day prefeeding period and a 5-day collection period. Two groups of four cows each were used. In each group a given forage constituted the sole feed.

The Venezuela grass was fertilized with 400 pounds of ammonium sulfate per acre and was cut between 40 and 60 days of age. The grass was cut daily at the field and chopped in a silage chopper prior to feeding.

[1] Research Assistant in Chemistry, and Head, respectively, Animal Husbandry Department, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R.

[2] Italic numbers in parentheses refer to Literature Cited p. 106.

The plantain pseudostalks fed were cut at the Corozal Substation farm when around 1 year old. They were brought in quantities sufficient to supply the requirement of this feed for 3 days. They were chopped into small pieces about 1 inch long with a machete.

The animals were fed three or four times during the day. The roughage given and refused was carefully weighed daily for each cow. All feces were carefully collected in a pail and weighed for each 24-hour period for 5 consecutive days. Cows were weighed every morning around 7:00 a. m. during the 5–day collection period.

During the collection period a 25-gm. sample of fecal material was collected twice daily, in the morning and in the afternoon. The samples were accumulated in the same jar to a total of 250 gm. throughout the collection period. A 500-gm. sample of roughage material was collected once a day for 5 days. Fecal and roughage samples were air-dried at room temperatures for 5 days for dry-matter determinations.

The dried samples were ground in a Wiley mill and placed in glass bottles. Crude protein determinations were made according to A.O.A.C. Official Methods (*1*). Calorimetric determinations were made according to the Parr oxygen-bomb combustion methods (*2*). A total of 14 roughage samples and 8 fecal samples was analyzed.

RESULTS AND DISCUSSION

Dry-matter, crude-protein, and gross-energy determinations for Venezuela grass, plantain pseudostalks, and fecal samples from both roughages are given in table 1.

Plantain pseudostalks were very much lower in dry-matter and crude-protein content than Venezuela grass. The two roughages were more or less equal as to gross-energy values.

The fecal samples from the plantain group were lower in dry-matter content. Both fecal samples had higher dry-matter values than the roughage samples.

The fecal samples had higher protein values than the roughage ingested. The higher values obtained for dry matter and crude protein in the feces can be explained in part by the high water content of the roughages fed.

Values for green-roughage and dry-matter consumption, and for fecal and dry-matter elimination are given in table 2. There was a marked difference in green-roughage consumption and a small difference in dry-matter intake which can be attributed to the differences in moisture content and palatability between the two roughages studied. The high moisture content of the plantain pseudostalks and the poor palatability of the Venezuela grass may be the reason for the low dry-matter intake.

Digestible-energy and digestible-protein values for Venezuela grass and

plantain pseudostalks are given in table 3. Two of the cows on Venezuela grass had a negative nitrogen balance and were eliminated in the calculation of the digestion coefficient for protein. These two cows did not eat

TABLE 1.—*Dry matter, crude protein, and gross energy for Venezuela grass and plantain pseudostalks, and fecal samples (dry basis), averages for 4 cows per group*

Forage	Roughages					Feces				
	Samples	Dry matter	Crude protein	Gross energy		Samples	Dry matter	Crude protein	Gross energy	
	Number	*Percent*	*Percent*	*Cals./gm.*	*Therms*	*Number*	*Percent*	*Percent*	*Cals./gm.*	*Therms*
Venezuela grass	7	17.68	6.41	3.62	1.642	4	21.30	8.96	3.62	1.642
Plantain pseudostalks	7	5.64	4.64	3.44	1.560	4	12.65	12.71	3.94	1.784

TABLE 2.—*Green roughage and dry matter consumed, and feces and dry matter eliminated per day, averages for 4 cows per group on Venezuela grass or plaintain pseudostalks*

Forage	Cows	Average roughage consumption per day	Average dry-matter consumption per day	Average fecal elimination per day	Average dry-matter elimination per day
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Venezuela grass	4	23.11	4.10	10.10	2.06
Plantain pseudostalks	4	117.97	6.72	13.38	1.66

TABLE 3.—*Digestible-energy and digestible-protein values for Venezuela grass and plantain pseudostalks, averages for 4 cows per group*

Forage	Cows	Digestible energy	Energy[1]		Digestible protein	Digestible coefficient
	Number	*Cals./lb. D.M.*	*Therms*	*Percent*	*Percent*	*Percent*
Venezuela grass	4	3,339.85	3.34	49.76	2.97	46.28
Plantain pseudostalks	4	7,523.18	7.52	71.76	1.53	32.93

[1] The digestible-energy values of plantain pseudostalks are higher than those of Venezuela grass.

enough of this grass. This confirms the fact that Venezuela grass is not palatable enough to the animals.

The protein in Venezuela grass seemed to be more digestible than that in the plantain pseudostalks. The Venezuela grass contained 6.41 percent of crude protein of which 46.28 percent was digestible, while the plantain pseudostalks contained 4.64 percent of protein, of which 32.93 percent was

digestible. Therefore, the percentage of digestible protein in Venezuela grass and plantain pseudostalks was 2.97 and 1.53, respectively.

SUMMARY

A study was undertaken to determine the digestibility of Venezuela grass (*Paspalum fasciculatum*) and plantain pseudostalks (*Musa paradisiaca*). Two groups of four cows were fed separately with the two roughages.

It was found that plantain pseudostalks were lower in dry matter, crude protein, and gross energy than Venezuela grass.

Based on feed consumption and dry-matter intake, plantain pseudostalks were more palatable than Venezuela grass.

The protein digestibility of Venezuela grass was higher than that of plantain pseudostalks. The reverse held for the energy digestibility.

RESUMEN

Se llevó a cabo un estudio para determinar la digestibilidad de la yerba Venezuela (*Paspalum fasciculatum*) y la de los seudo-tallos del plátano (*Musa paradisiaca*). Dos grupos de 4 vacas cada uno fueron alimentados separadamente con estos dos forrajes.

Se encontró que el contenido de los seudo-tallos del plátano es bajo en materia seca, proteína cruda y en valores de energía, al compararse con el contenido de la yerba Venezuela.

Los seudo-tallos del plátano fueron más apetecibles para el ganado que la yerba Venezuela, según fué comprobado por el consumo diario de ambos forrajes y por la cantidad de materia seca ingerida de cada uno.

La digestibilidad de la proteína de la yerba Venezuela fué mayor que la de los seudo-tallos del plátano. En cuanto a la energía digerible fué todo lo contrario, esto es, mayor para los seudo-tallos del plátano y menor para la yerba Venezuela.

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The Yields and Composition of Five Grasses Growing in the Humid Mountains of Puerto Rico, as Affected by Nitrogen Fertilization, Season, and Harvest Procedures[1]

Ruben Caro-Costas, José Vicente-Chandler, and Jacinto Figarella[2]

INTRODUCTION

Napier grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*), Para grass (*Panicum purpurascens*), Pangola grass (*Digitaria decumbens*), and molasses grass (*Melinis minutiflora*), are among the most widely used forages of the humid Tropics. However, little is known of their relative productivity under different conditions as affected by fertilization, season of the year, and harvest procedure.

In Puerto Rico, Rodríguez (4)[3] found that applications of 200 pounds of nitrogen per acre yearly about doubled yields of Napier, Guinea, and Para grasses in the humid coastal area. Under similar conditions, Vicente, Silva, and Figarella (*8*) found that Napier, Guinea, and Para grasses responded in yield to nitrogen applications up to 800 pounds per acre yearly. Protein content and yields of the grasses increased sharply with nitrogen rates up to 1,600 pounds per acre yearly. About half of the fertilizer nitrogen was recovered in the forage at all rates up to 800 pounds per acre yearly, but pounds of dry matter produced per pound of nitrogen decreased beyond the 400-pound level. Phosphorus content of the forage decreased, lignin content increased, while the calcium, potassium, and magnesium contents were not consistently affected by increasing nitrogen rates. There were marked seasonal variations in yield. Rivera-Brenes (*3*) found that Napier produced more green forage than Guinea or Para grasses in this area.

Vicente and Figarella (*7*) found that the application of 100 pounds of nitrogen per acre to unirrigated Guinea grass growing in the semiarid South coast increased yields by about one-third. Vicente, *et al.* (*6*) reported that applications of 300 pounds of nitrogen per acre yearly more than doubled yields of Guinea grass growing with irrigation in this region. Under similar conditions, Little, Vicente, and Abruña (*2*) found that yields of irrigated

[1] This report covers work carried out cooperatively between the Soil and Water Conservation Research Division, Agricultural Research Service, USDA, and the Agricultural Experiment Station of the University of Puerto Rico.

[2] Agronomist (cooperative between the above agencies), Project Supervisor, and Soil Scientist, Soil and Water Conservation Research Division, Agricultural Research Service, USDA, respectively, stationed at the Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R.

[3] Italic numbers in parentheses refer to Literature Cited, pp. 119–20.

Napier, and Guinea grasses increased with nitrogen rates up to 800 pounds per acre yearly, while Pangola grass responded little to applications of nitrogen in excess of 200 pounds. Protein content of the forage increased with nitrogen rates up to 1,600 pounds per acre yearly. Highest yields of dry matter were produced by Napier, followed by Guinea and Pangola grasses, in that order.

The experiment herein reported was carried out to determine the productivity of Napier, Guinea, Para, Pangola, and molasses grasses, as affected by nitrogen fertilization and season of the year, with both cutting and simulated grazing management under conditions typical of the steep humid mountains of Puerto Rico.

MATERIALS AND METHODS

The experiment was carried out at Orocovis, P.R., which has a mean annual temperature of about 75°F., and a seasonal variation of less than 10°F. Annual rainfall was 76 and 91.8 inches, respectively, for the 2 years of experimentation. The experiment was carried out on Catalina clay on a 30-percent slope. The surface 6 inches contained 4.5 percent of organic matter; it had a pH of 5.3, 16.5 m.e. of exchange capacity, and 10.4 m.e. of exchangeable bases per 100 gm. of soil, a volume weight of 0.98 with 11 percent of the pores drained at pF 1.78, and a saturated percolation rate of about 6 inches per hour.

TREATMENTS

Grasses used: Napier, Guinea, Pangola, molasses, and Pará with some Carib grass (*Eriochloa polystàchya*).

Nitrogen rates: 0, 200, 400, and 800 pounds N per acre yearly as ammonium sulfate in 6 or 9 equal applications, depending on frequency of harvest.

Harvest procedure: 1, Cut every 60 days; 2, simulated grazing every 40 days.

METHODS

Plots with cutting management were harvested every 60 days by cutting the grasses close to the ground. Simulated grazing was obtained by a combination of cutting and plucking once every 40 days, in an attempt to produce a condition comparable to that on companion plots intermittently grazed by livestock.

All combinations of treatments were tested using a split-plot design with harvest procedure as the main plots, grasses as the subplots, and nitrogen rates as the sub-subplots. The latter were $\frac{1}{200}$ of an acre in size, surrounded by ditches to prevent fertilizer from washing into adjoining plots. All treatments were replicated four times.

The upper 6 inches of soil in all plots was limed to a pH of about 6.5 and relimed annually to this level. Blanket additions of phosphorus and potassium were made at the rate of 200 pounds of P_2O_5 from 20-percent superphosphate per acre in one annual application, and 400 pounds of K_2O from KCl per acre yearly in six or nine equal applications, depending on frequency of harvest.

The forage from all plots at every harvest was weighed and analyzed for dry matter and total nitrogen. The crude protein was calculated using the factor 6.25 × N. Samples from all cuttings of the second year were composited by plots and analyzed for calcium, phosphorus, potassium, magnesium, lignin, and "digestible laboratory nutrients", using the method of Thurman and Wehunt (*5*). Residual yields were taken over a 60-day period following termination of the experiment.

RESULTS

YIELDS OF FORAGE

The grasses gave about the same response to nitrogen fertilization in both years. The check plots were lower the second year, however, showing that the soil nitrogen had been depleted. Relative productivity of the grasses and effects of fertilization, harvesting procedure, and season were very similar during both years.

The data in table 1 show that molasses grass yielded much less than the others with both systems of harvesting when nitrogen was applied. With cutting management, Napier, Guinea, Pangola, and Para grasses yielded about the same at all nitrogen levels, except at the 800-pound rate, at which Napier produced about 20 percent more forage than the others. At the 400-pound nitrogen rate all these grasses yielded about 24,000 pounds of dry matter per acre yearly. With simulated grazing management, however, Guinea outyielded all the grasses at all nitrogen levels. Pangola, Napier, and Para grasses produced similar yields at all nitrogen levels.

These data and figure 1 show that all grasses, except molasses, responded in yield to nitrogen applications of up to 800 pounds per acre yearly, with both cutting and simulated grazing management. However, except with Napier harvested by cutting, and Guinea harvested by grazing, increases in yield fell off sharply beyond the 400-pound level. Yields of these four grasses were doubled or more by the application of 400 pounds of nitrogen per acre yearly. Molasses grass, on the other hand, gave only a moderate response to the application of 200 pounds of nitrogen, and was little affected by heavier applications.

All grasses yielded less with simulated grazing than with cutting management. With 400 pounds of nitrogen, yields of Para, Napier, and Pangola grasses were lowered 35, 40, and 31 percent, respectively, by simulated

grazing, but those of Guinea and molasses grasses dropped only 16 and 20 percent, respectively. These decreases in yield were caused by the shorter harvest interval (*8*) as well as by the higher cutting in the grazed plots as discussed later.

TABLE 1.—*The effect of nitrogen fertilization and 2 methods of harvesting on yields and protein contents of 5 tropical grasses, and on the efficiency of nitrogen utilization over a 2-year period*

Grass and nitrogen (pounds) per acre yearly	Grasses harvested by cutting every 60 days				Grasses harvested by simulated grazing every 40 days			
	Dry forage per acre yearly	Protein	Recovery of fertilizer N	Dry forage per pound increment of N	Dry forage per acre yearly	Protein	Recovery of fertilizer N	Dry forage per pound increment of N
	Lb.	*Percent*	*Percent*	*Lb.*	*Lb.*	*Percent*	*Percent*	*Lb.*
Para grass								
0	8,742	6.4	—	—	6,693	8.2	—	—
200	19,638	6.6	58.6	54.5	12,179	8.8	42.0	27.4
400	24,025	8.0	55.0	21.9	15,694	10.0	41.0	17.6
800	26,054	9.9	40.3	5.1	16,479	12.9	31.5	1.9
Guinea grass								
0	9,675	7.7	—	—	8,821	8.7	—	—
200	19,645	7.6	60.2	49.9	15,176	8.8	45.6	31.8
400	23,002	7.8	42.3	16.8	19,330	9.4	42.0	20.8
800	28,180	10.0	41.5	12.9	26,696	11.4	45.3	18.4
Napier grass								
0	13,308	7.4	—	—	7,579	10.5	—	—
200	21,395	6.9	39.9	40.4	11,591	10.6	35.2	20.1
400	25,214	7.5	36.3	19.1	14,953	11.6	37.4	16.8
800	33,284	8.7	38.5	20.2	19,415	12.8	33.7	11.2
Pangola grass								
0	8,614	6.4	—	—	6,981	7.0	—	—
200	18,106	6.9	55.8	47.3	11,287	8.7	40.5	21.5
400	23,707	7.9	53.0	28.0	16,332	10.3	47.9	25.2
800	26,686	10.9	47.1	7.4	18,362	12.0	34.4	5.1
Molasses grass								
0	9,275	7.3	—	—	6,479	7.9	—	—
200	13,271	8.1	32.0	20.0	8,880	8.4	18.7	12.0
400	13,246	9.8	25.0	—	10,647	10.1	22.6	8.8
800	11,937	11.2	13.2	—	8,863	11.8	10.8	—

Figure 2 shows that season of the year had a marked and very similar influence on yields of all grasses with lowest productivity during the winter months of December through March. Napier grass produced more forage than the others during the last months of both years.

Table 2 shows that seasonal variations in yield generally increased

sharply with nitrogen rates up to 400 pounds per acre yearly. However, the variation was not further accentuated by raising rates to 800 pounds of nitrogen.

Figures 3 and 4 show that season of the year also strongly affected the response of the grasses to nitrogen fertilization with both simulated grazing and cutting management. Rate of yield increases with nitrogen fertilization were much lower during the winter months. Molasses grass generally re-

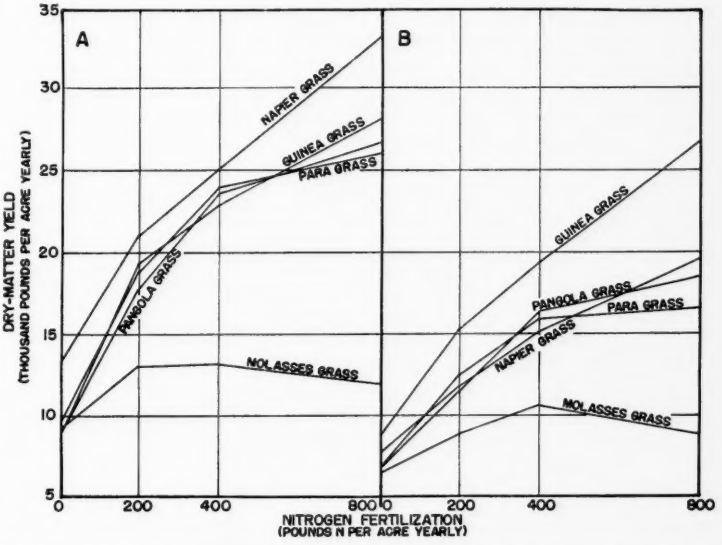

FIG. 1.—The effects of nitrogen fertilization on yields of 5 grasses in the humid mountains of Puerto Rico when cut every 60 days (A) and grazed every 40 days (B).

sponded only to the 200-pound rate of nitrogen at all seasons. The other grasses generally responded well to the 200-pound level during the winter months of slow growth, while responding strongly to at least the 400-pound level during the remainder of the year.

It is important to note in figure 4 that the superiority in yields of Guinea grass with grazing management was attained during seasons of fast growth. There was no great difference in the productivity of Napier, Guinea, Para, and Pangola grasses during seasons of slow growth when forage is at a premium.

Little or no weeding was required with heavy nitrogen applications, but weeds were a constant problem in the no-nitrogen plots.

PROTEIN CONTENTS AND YIELDS

Table 1 shows that all the grasses had a remarkably similar protein content when treated alike. For example, with cutting management and 400

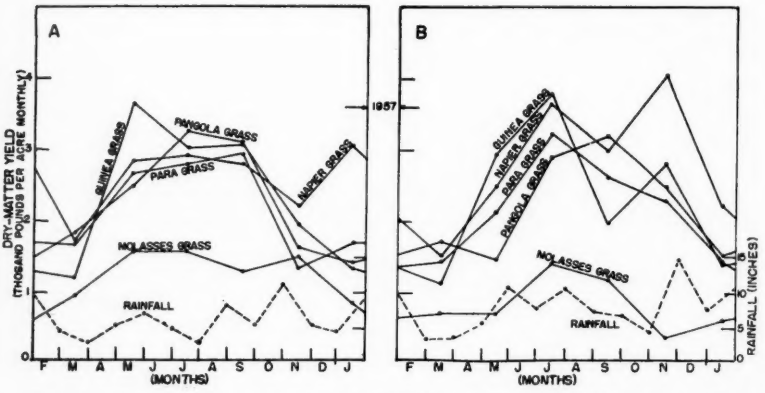

FIG. 2.—The seasonal productivity in 1956 (A) and 1958 (B) of heavily fertilized grasses cut every 60 days over a 2-year period in the humid mountain region of Puerto Rico.

TABLE 2.—*The effect of nitrogen rates (pounds) on seasonal variations in yields of 5 grasses expressed as percentage by which winter yields were lower than yields during the remainder of the year*

Grass	Effect of indicated pounds N per acre yearly		
	0	200	400
Para	0	26	35
Guinea	19	42	50
Napier	23	23	31
Pangola	0	31	40
Molasses	21	30	29
Average	12.6	30.4	37

pounds of nitrogen per acre yearly, the protein contents of Para, Guinea, Napier, and Pangola grasses varied only from 7.5 to 8.0 percent. The slightly higher protein content of molasses grass is attributed to the concentrating effect of lower yields in the presence of an equal supply of nitrogen.

Protein content of all grasses increased with nitrogen rates, with the

highest increases occurring beyond the 200-pound level. With cutting management, protein content increased from an average of 7 percent when no nitrogen was applied to 10.1 percent when 800 pounds of nitrogen were applied.

Protein content of the forage was considerably higher when the grasses were harvested by simulated grazing, largely due to the shorter harvest nterval used.

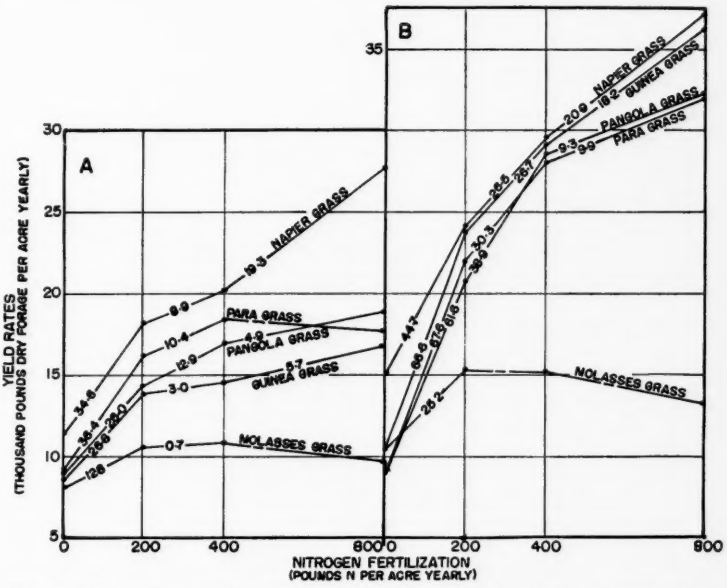

FIG. 3.—The responses of 5 grasses to nitrogen fertilization during seasons of slow—approximately December through March (A), and fast—remainder of the year (B), growth when harvested by cutting every 60 days. Numbers show pounds of dry forage per pound increment of N.

Season of the year had a marked and very similar influence on the protein content of all grasses, with highest contents occurring when yields were lowest during the winter months and vice versa. With cutting management and 800 pounds of nitrogen, for example, protein content of the grasses varied from as low as 6.9 to as high as 15.6 percent.

EFFICIENCY OF NITROGEN UTILIZATION

Table 1 shows that lowest recovery of fertilizer nitrogen in the forage was with molasses grass, with Napier grass recovering less nitrogen than the

others at the lower rates. Recovery of nitrogen tended to be lower when the grasses were harvested by simulated grazing and to decrease slightly with increasing nitrogen rates. At the 400-pound rate of nitrogen an average of about 40 percent of the fertilizer nitrogen was recovered in the forage.

There were no marked over-all differences in the pounds of forage pro-

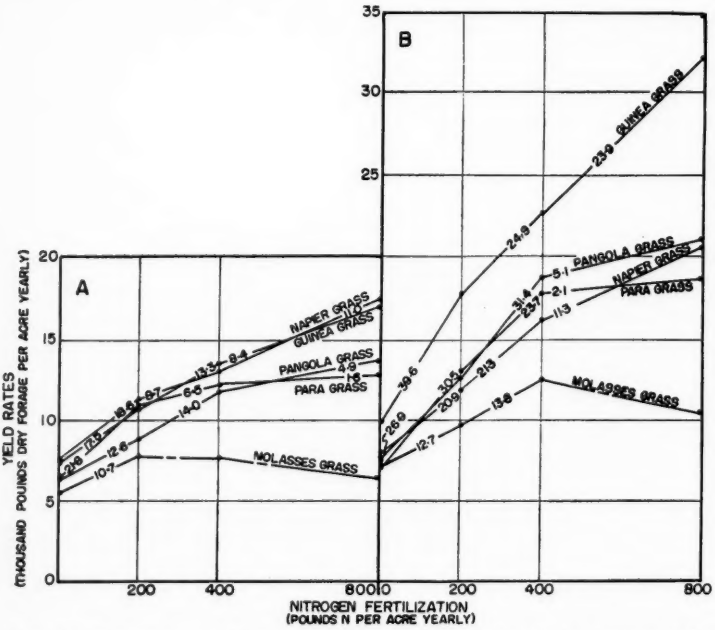

FIG. 4.—The responses of 5 grasses to nitrogen fertilization during seasons of slow—approximately December through March (A), and fast—remainder of the year (B), growth with grazing management. Numbers show pounds of dry forage per pound increment of N.

duced per pound of nitrogen by the highest yielding grasses at rates of 400 pounds or less per acre (table 1). Molasses grass produced much less forage per pound of nitrogen at all rates than did the other grasses. With all grasses, forage produced per pound of nitrogen decreased rapidly with increasing nitrogen rates. Para grass, for example, produced 54.5, 21.9, and 5.1 pounds of forage per pound of nitrogen at the 200-, 400-, and 800-pound rates, respectively. Less forage was generally produced per pound of nitrogen with grazing than with cutting management.

Season of the year markedly affected the efficiency of nitrogen utilization

as shown in figures 3 and 4, with more dry matter produced per pound of fertilizer nitrogen during seasons of fast growth. For example, with cutting management, the second 200 pounds of nitrogen with the four highest yielding grasses produced an average of only 8.8 pounds of forage per pound of nitrogen in winter, compared with 30.8 pounds during the remaining 8 months of the year.

FORAGE COMPOSITION

Dry-matter content of the grasses, except Napier which had only about 19 percent, varied between 23 and 27 percent.

Table 3 shows the composition of the various grasses when fertilized with

TABLE 3.—*The mineral composition and lignin content of 5 tropical grasses when heavily fertilized*[1] *and cut every 60 days*[2]

Grass	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Lignin	D.L.N.[3]
Para	1.28	0.18	2.20	0.48	0.33	8.41	54.9
Guinea	1.25	.19	2.36	.65	.43	9.43	54.8
Napier	1.20	.25	2.78	.38	.25	8.81	54.4
Pangola	1.26	.20	2.32	.46	.28	8.91	54.4
Molasses	1.57	.24	2.24	.43	.33	9.81	55.7

[1] 400 lb. N; 200 lb. P_2O_5; and 400 lb. K_2O per acre yearly in 6 equal applications.

[2] All values are in percentages on a dry-weight basis and are averages of 4 replicate samples composited yearly from all cuttings.

[3] Digestible laboratory nutrients determined by the method of Thurman and Wehunt (*5*).

400 pounds of nitrogen per acre yearly and harvested by cutting every 60 days. Guinea grass had a markedly higher calcium and magnesium content than the others. Otherwise, the grasses were similar in mineral and lignin content and in "digestible nutrients" (D.L.N.).

DISCUSSION

The high yields of forage produced on this steep, eroded soil are worthy of note. Yields and responses of the grasses to nitrogen are very similar to those on the north coast (*8*). With cutting management and 400 pounds of nitrogen, all grasses, except molasses, yielded about 24,000 pounds of dry matter, or 50 to 60 tons of green forage, per acre yearly, containing about 8 percent of protein. This is enough to feed about five steers per acre. With simulated grazing management and 400 pounds of nitrogen, Guinea grass yielded about 19,000 pounds of dry matter per acre yearly, compared to about 15,000 pounds for Pangola, Para, and Napier grasses. It is interest-

ing to note that in a nearby grazing experiment, well-fertilized Guinea grass pastures produced about 1,200 pounds gain-in-weight per acre yearly when grazed by young dairy heifers, compared to about 950 pounds produced by Pangola and Napier grass pastures.[4]

The lower yields obtained with grazing compared to cutting management, can be explained by the shorter harvest interval (*8*) as well as by trampling losses. Guinea grass was probably least affected by shortening the harvest interval since Vicente, *et al.* (*8*), found that yields of Napier were reduced about 45 percent, those of Para about 23 percent, but those of Guinea grass only 16 percent, when the harvest interval was shortened from 60 to 40 days. The approximately 8 inches of growth left on the ground also favored Guinea and molasses grasses over the others.[4]

The similarity in yields, protein content, response to nitrogen fertilization and seasonal growth rates by Napier, Para, Pangola, and Guinea grasses under these conditions is striking. Although molasses grass clearly yields less than the others, the difference was accentuated in this experiment by its sensitivity to cutting and to burning by fertilizer, which reduced its stand appreciably.

The slow growth during winter was frequently associated with low rainfall, but yields were also low during this period even when rainfall was abundant, as occurred in January of 1956 and 1958. On the other hand, the low rainfall in June and July of 1956 did not affect yields. Apparently, the shorter days and cooler weather of winter reduce growth and stimulate early flowering of these grasses.

Nitrogen fertilization accentuated seasonal variations in yield by increasing growth only moderately during winter, while stimulating it tremendously during the remainder of the year.

Assuming that 15 pounds or more of dry forage must be produced per pound of fertilizer nitrogen, it appears from table 1 that with both systems of management, the application of 400 pounds of nitrogen per acre yearly to all grasses except molasses, was warranted. However, figure 3 shows that only the 200-pound rate was warranted during the winter months of slow growth, while it paid to apply at least 400 pounds of nitrogen to these four grasses during the remainder of the year.

By using the 200-pound rate in winter and the 400-pound rate during the remaining months, only about 330 pounds of nitrogen would be used yearly, yet, annual yields would be similar to those obtained with the 400-pound rate, as shown in table 4. However, these data show that such a practice further accentuates seasonal variations in yield and would be desirable only when the number of animals can be seasonally adjusted, or

[4] Authors' unpublished data.

where forage can be stored as hay or silage, which is usually impractical on the steep slopes of the humid mountain region.

On the other hand, these data show that seasonal variations can be greatly decreased by using the 400-pound rate of nitrogen to obtain maximum yields during the four critical winter months and the 200-pound rate to reduce summer growth. With such a system, only about 265 pounds of nitrogen are used per acre yearly, and an additional 50 pounds or so may be saved by counting on residual effects of the heavy winter applications (*8*) and not fertilizing early in spring. Yet, total yields are cut only about

TABLE 4.—*The approximate effect of 3 systems of nitrogen fertilization on yields and seasonal variations in growth of 5 grasses cut every 60 days*

Grass	Yields in pounds of dry forage per acre yearly			Seasonal variations in yield as percentage of winter yields lower than summer		
	A[1]	B[2]	C[3]	A[1]	B[2]	C[3]
Para	24,000	24,200	20,800	35	46	17
Guinea	23,000	24,100	20,800	50	52	39
Napier	25,200	24,400	22,500	31	37	15
Pangola	23,700	23,900	19,500	40	50	18
Molasses	13,300	13,600	13,700	29	28	29
Average	21,850	22,040	19,460	37	42.6	23.6

[1] A, 400 lb. N per acre yearly in 6 equal applications.

[2] B, 300 lb. N in 2 winter applications at the 200-lb. rate, and 4 summer applications at the 400-lb rate.

[3] C, 265 lb. N in 2 winter applications at the 400-lb. rate and 4 summer applications at the 200-lb. rate.

20 percent, and this occurs during the season when forage is abundant. About 20,000 pounds of dry matter would be produced per acre yearly with this system by all grasses, except molasses.

Seasonal variations in yield could be further reduced by using a longer harvest interval to increase yields in winter and a shorter one to reduce summer yields (*8*). Such a practice would also reduce seasonal variations in protein content since the depressive effect of the longer harvest intervals in winter would be counteracted by the heavier nitrogen applications, while the shorter harvest interval in summer would help to compensate for the lower nitrogen rates.

Several variations in yield could also be induced by grazing in summer while using a lower nitrogen rate and cutting in winter while using a higher nitrogen rate.

The higher protein content of the grasses during seasons of slow growth, the slightly higher protein content of the lower yielding molasses grass, and the higher protein content with grazing management, are explained by the concentrating effect of lower yields in the presence of a relatively constant amount of nitrogen in the soil.

SUMMARY

The productivity of Napier, Guinea, Para, Pangola, and molasses grasses, as affected by nitrogen fertilization and season of the year under both cutting and simulated grazing management, were determined under conditions typical of the humid mountain region of Puerto Rico.

Molasses grass yielded much less than the others, all of which were very similar in yield, response to nitrogen fertilization, and seasonal growth rates with both harvest procedures, except that Guinea grass outyielded the others with grazing management. With cutting management and 400 pounds of nitrogen per acre yearly, Napier, Guinea, Para, and Pangola grasses yielded about 24,000 pounds of dry matter per acre yearly having about 8 percent of protein. Yields were lower with grazing than with cutting management.

Season of the year markedly affected growth of all grasses with lowest yields occurring from December through March. Seasonal variations were accentuated by nitrogen fertilization.

Annual yields of the Para, Guinea, Napier, and Pangola grasses increased rapidly with nitrogen rates up to 400 pounds. However, response was generally limited to the 200-pound rate during seasons of slow growth, but was strong up to at least the 400-pound rate at other times. Molasses grass responded only to the application of 200 pounds of nitrogen per acre yearly at all seasons.

Protein content of all the grasses was similar, increased with nitrogen rates, was higher during seasons of slow growth, and lower with cutting than with simulated grazing management. Napier grass had the lowest dry-matter content, while Guinea grass had a markedly higher calcium and magnesium content than the others. Otherwise, the forages were very similar in mineral and lignin content.

There was little difference in efficiency of nitrogen utilization by the highest yielding grasses. Efficiency was lower with grazing management, higher during seasons of fast growth, and decreased with increasing nitrogen rates.

RESUMEN

Se estudió la productividad de las yerbas Napier, Guinea, Pará, Pangola y Melao, con distintos niveles de nitrógeno, durante distintas épocas del

año, cosechándose por corte y pastoreo simulado bajo condiciones típicas de la región montañosa de Puerto Rico.

La yerba Melao tuvo un rendimiento menor que las otras, las cuales tuvieron una producción similar y respondieron al nitrógeno en forma similar. La yerba Guinea produjo más que las otras en pastoreo simulado. Cuando se cosecharon por corte y se les aplicó 400 libras de nitrógeno por cuerda anualmente, las yerbas Napier, Guinea, Pará, y Pangola produjeron alrededor de 24,000 libras de forraje seco por cuerda por año, con un 8 por ciento de proteína. Las yerbas produjeron menos cuando se cosecharon simulando el pastoreo que cuando se cortaban.

La época del año afectó el crecimiento de las yerbas, las cuales produjeron los rendimientos más bajos en los meses de diciembre a marzo. Las aplicaciones de nitrógeno acentuaron la variación en el crecimiento durante las distintas épocas del año.

El rendimiento de las yerbas Pará, Guinea, Napier y Pangola aumentó rápidamente según se aumentaban los niveles de nitrógeno, por lo menos hasta 400 libras de nitrógeno por cuerda por año. Este aumento fué más notable durante las épocas de crecimiento rápido. La yerba Melao respondió sólo a la aplicación de 200 libras de nitrógeno por cuerda por año.

El contenido de proteína de todas las yerbas fué similar. El contenido de proteína aumentó según aumentaba el nivel de nitrógeno; subió durante épocas de crecimiento lento, y bajó durante épocas de crecimiento rápido; y fué más bajo cuando se cortaba la yerba que cuando se simuló el pastoreo. La yerba Napier tuvo el contenido más bajo de materia seca y la Guinea el más alto de calcio y magnesio.

Las cuatro yerbas de producción más alta difirieron poco en su eficiencia para utilizar el nitrógeno. La eficiencia de utilización de este nutriente fué menor bajo el pastoreo simulado, subió durante la época de crecimiento rápido y bajó según aumentaba el nivel de nitrógeno.

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Effects of Irrigation, Nitrogen Levels, and Plant Population on Corn Yields in Lajas Valley, P. R.[1]

Roberto Vázquez[2]

INTRODUCTION

There are a number of factors that influence crop yields. Among the most important are soil moisture, soil fertility, and plant population. The optimum level of moisture for plant growth is very important in irrigation agriculture, because it is fundamental in determining the time for, and the amount of, irrigation.

Little is known of the relationship between water used by corn and the corresponding growth and yield on soils and under climatic conditions of Puerto Rico. Some studies have been made in the United States by Boswell, Anderson, and Stacey (*2*)[3] who found a response to nitrogen and plant population when irrigation was applied. However, data of this kind are greatly affected by climatic and soil conditions and can only be applied to closely similar areas.

A response of corn to irrigation was observed in previous limited studies at Lajas Substation. However, in view of the impending development of additional water supply for the Lajas Valley, it was decided to undertake a series of experiments at the Lajas Substation to evaluate more thoroughly the effect of irrigation in combination with nitrogen fertilizer and different plant populations on the production of field corn.

EXPERIMENTAL PROCEDURE

Three experiments were conducted at Lajas Substation farm. The first experiment was planted July 12, 1956 and harvested November 5, 1956; the second was planted March 14, 1957 and harvested July 23, 1957; the third was planted April 17, 1958 and harvested August 17, 1958. The experiments were located in different sites of the farm and a simple lattice design was used throughout. The treatments included a combination of 3

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[2] Assistant Irrigation Scientist, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R. The author wishes to thank Lyman S. Willardson, former Irrigation Engineer of Lajas Substation, for his valuable assistance in the planning of these experiments and Raúl Pérez Escolar, Assistant Soil Scientist, for his help in obtaining the soil-moisture release data.

[3] Italic numbers in parentheses refer to Literature Cited, p. 137.

irrigation levels, 3 nitrogen levels, and 3 plant-population levels, with 4 replications, there being a total of 108 plots. The plant-population fertilizer-treatment combinations were grouped in blocks of 9 plots under each irrigation treatment, there being 3 blocks within each replication. The plots were 6 rows, 18 feet wide and 18 feet long. Six guard rows were left on each side of the experiment. The entire experiment covered an area of about 240 feet wide by 200 feet long.

The soil in the different sites was classified as Santa Isabel clay. This is one of the predominant soil types in Lajas Valley. No crops had been planted in these sites during the last few years. The soil moisture retained

TABLE 1.—*Moisture-release data (percentage by weight) for Santa Isabel clay soil, Lajas, P. R.*

Moisture tension (atmospheres)	Soil moisture at—			
	Site No. 1 at—		Site No. 2 at—	
	Soil depth of 3–6 inches	Soil depth of 18–21 inches	Soil depth of 3–6 inches	Soil depth of 18–21 inches
0.1	41.3	43.7	31.2	31.4
.3	38.3	39.7	30.5	30.4
.6	37.2	37.3	28.5	30.0
1.0	35.8	35.2	26.8	29.8
2.0	34.3	34.0	26.1	29.6
15.0	21.4	22.7	16.2	16.1
Bulk density[1]	1.03	1.24	1.33	1.48

[1] Grams per cubic centimeter.

at different tensions and other physical properties were determined (table 1).

IRRIGATION

The three following irrigation treatments were tried: Frequent, irrigated when 20 percent of the available moisture had been depleted from the active root zone; intermediate, irrigated when 60 percent of the available moisture had been depleted from the active root zone; no irrigation, not irrigated after the crop was established.

The soil was plowed and harrowed several times until a good seedbed was obtained. Furrows about 4 inches deep and 3 feet apart were made in the whole experiment for the purpose of irrigation. Feeding ditches were made at the head of each irrigation block and used as equalizing bays by means of plastic dams placed at the end of each block. Plastic siphon tubes

were used as flow controls for each furrow. A heavy irrigation was applied to the whole area after being planted in each experiment except in experiment No. 3 in which there was enough soil moisture to establish the plants. Subsequent irrigations were made according to the treatments involved.

PLANT POPULATION

The 3 plant-population levels tried were 9,600, 14,500, and 19,400 plants per acre. In experiment No. 3 the plant-population treatment of 9,600 was substituted with 24,900 plants per acre. A field-corn variety, Mayorbela, was planted in the top of the furrows at a 3-foot distance between furrows, with 9, 12, and 18 inches between plants. In experiment No. 3 the 18-inch distance was changed to a 7-inch one, corresponding to the 24,900 plant-population treatment. Two seed were placed in each hill; thinning to one plant per hill was done after the plants emerged.

FERTILIZER

The nitrogen-fertilizer treatments used were 0, 80, and 160 pounds per acre and were applied when the corn plants were about 8 inches high. Nitrogen in the form of ammonium sulfate (20.5 percent of N) was applied at the rates of 80 and 160 pounds per acre. Phosphorus and potassium in the form of superphosphate (20.5 percent of P_2O_5), and muriate of potash (61 percent K_2O), respectively, were applied to the whole experiment at the rate of 200 pounds each of P_2O_5 and K_2O per acre. All the fertilizer was placed in a small furrow opened about 3 inches at both sides of the corn plant.

SOIL-MOISTURE CONTROL

Disturbed and undisturbed soil samples were taken from 3 to 6 inches and from 18 to 21 inches deep at both sites where the experiments were conducted. The soil moisture retained by the undisturbed soil samples at different tensions in the low-tension range was determined in the pressure plate. The moisture retained at higher tensions by the disturbed soil samples was determined in the pressure membrane apparatus (*5*). Bulk-density determinations were made at both sites at each depth.

Tensiometers (*4*) were installed in the highest fertility and plant-population plots of the frequently irrigated blocks at 6-, 12-, 18-, and 24-inch depths. Two groups of tensiometers were placed on the top of the forrow, close to the corn plants. Readings of the instruments were taken every day. Irrigation of the frequently irrigated plots were made when the average soil-moisture tensions in the active root zone became 750 cm. of water. Gypsum resistance-blocks of the Bouyoucos type (*1*) and home-made models were installed in experiment No. 3 at 6-, 12-, 18-, 24-, and 30-inch depths in the frequently, intermediately, and nonirrigated plots.

The irrigations of the intermediately irrigated plots were made when the average available soil moisture in the active root zone dropped to 28.8 percent in experiments Nos. 1 and 2. In experiment No. 3 the soil was a little different, having a different soil-moisture retention; the average percentage was found to be 22.2 for irrigation of the intermediately irrigated plots. About 2 inches of water were applied whenever an irrigation was carried out (table 2). Soil samples for moisture determination were taken during

TABLE 2.—*Irrigation frequencies used in the corn experiment at Lajas Substation*

Moisture treatment	Dates of irrigation for—		
	Experiment No. 1, 1956	Experiment No. 2, 1957	Experiment No. 3, 1958
No irrigation	July 16–17	Mar. 19	—
Intermediate irrigation	July 16–17 Aug. 24 Sept. 11	Mar. 19 Apr. 5 Apr. 20 May 10–11 May 27–28 June 13 July 5–6	May 19
Frequent irrigation	July 16–17 Aug. 11 Aug. 24 Sept. 3 Sept. 11 Sept. 27	Mar. 19 Apr. 5 Apr. 20 Apr. 30 May 10–11 May 17 May 27–28 June 13 June 22 July 5–6	May 16 May 27 June 20 July 18

the whole growing season to indicate the irrigation of the intermediately irrigated plots and to calculate the moisture extracted from the root zone in each moisture treatment.

In experiment No. 1 the soil samples were taken with an Uhland soil sampler at the following depths: 0 to 9, 9 to 15, 15 to 21, 21 to 27, 27 to 33, and 33 to 39 inches. In experiments Nos. 2 and 3 duplicate soil samples were taken with a soil auger at 3 depths: 3 to 9, 15 to 21, and 27 to 33 inches. The soil samples were taken twice a week in the plots having a combination of 9,400 plants and no nitrogen; 14,500 plants and 160 pounds of nitrogen; and 19,400 plants with 160 pounds of nitrogen per acre under each irriga-

tion treatment. In experiment No. 3 the soil samples were taken in the plots having 14,500, 19,400 and 24,900 plants per acre, respectively.

All samples were weighed and placed in an oven for 24 hours at 105°C. and the percentage of moisture on an oven-dry weight basis was determined. The water extracted from the top 3 feet of soil in the frequently and intermediately irrigated, and in the nonirrigated plots, was calculated for short periods throughout the whole growing season. A total of six samples at each moisture treatment was used to calculate the water extracted in those short periods and the whole growing season. The consumptive use of water was calculated by adding to the water extracted the rainfall during those periods.

CULTURAL PRACTICES

Weedings were made as was necessary to maintain the plots free of weeds. Every 10 days the whole experiment was sprayed with DDT (50-percent wettable) at the rate of 4 pounds per 100 gallons of water to control worm attack. When the kernels were dry, the central 12-foot-long sections of the central four rows of each plot were harvested. Percentage moisture of the kernel was determined.

EXPERIMENTAL RESULTS

An evaluation was made of the soil-moisture data and the effects of irrigation, nitrogen, and plant population on corn yields.

SOIL MOISTURE

As shown in table 1 there was a difference in the soil moisture retained at different tensions between the soil samples taken at sites Nos. 1 and 2. There was a difference in the soil moisture retained between the samples taken at 3-, to 6-, and 18- to 21-inch depths at each site.

Table 3 shows the rainfall data by days and months during the years 1956, 1957, and 1958, and the 10-year monthly average at Lajas Substation. As can be seen, 1957 was very dry, while 1958 had an abnormal rainfall distribution. Because of frequent rainfall it was not possible to calculate the water extracted from the root zone during the growing period of experiment No. 1. In some instances in experiment No. 2 the irrigations could not be performed as indicated by the soil-moisture measuring devices because of scarce irrigation water.

The consumptive use of water during short periods and throughout the whole growing season under each moisture treatment for experiments Nos. 2 and 3 is presented in tables 4 to 9. As shown in tables 4, 5, and 6, the total consumptive use in 125 days under experiment No. 2 varied from 19.99 inches in the frequently irrigated plots to 18.51 and 8.91 inches in

TABLE 3.—*Inches of daily rainfall during the growth period of each corn-yield experiment at Lajas, P. R., 1956–58*

Date	1956					1957					1958				
	Jul.	Aug.	Sept.	Oct.	Nov.	Mar.	Apr.	May	June	Jul.	Apr.	May	June	Jul.	Aug.
1		0.23		1.47		0.01							0.35	0.76	
2			0.17	.70	0.90	.12	0.08					0.05	.13	1.00	1.23
3			.45	.46	3.87				0.01			.28	.65		.35
4				.79					.02	0.02		.46			
5				.06								.11	.21	.26	2.49
6										.02	0.07	.26	.08	.26	.01
7												.02	1.42	.89	
8		.15					.03								.07
9	0.08	.04		.17								.24	.08	.49	.11
10				.41	.22	.01					.22		.01		.23
11			.02								.07				.21
12		.91	.02					0.26	.21			.03	.80		.07
13		.02	.10			.01		.02			.06	.04			.07
14					.09		.09				.02				
15		.03					.01				.07		.06		
16	.05		.65				.03	.22	.04					.15	.21
17				.78		.15									.05
18	.06		.08	.12					.17		.59	.02			
19	.12	.48	.45	.55			.11		.13		1.25	.10			
20	.45						.63				1.22		.61	.27	.02
21	2.65		.01	.65				.08						1.68	
22		.05		1.61				.18			.23		.04	.03	
23			.23								.12				
24		1.31	.03				.07	.03	.25	.06	.02		.07	.69	.02
25	.07			.03			.13	.38	.03		.68		.01		
26		.23	.44	.18	.19		.01								
27	.05				.62									.10	
28			.77	.85	.10	.01		.20				.36		.07	.56
29		.06		.11			.01	.01						.01	
30			1.43	1.18				.02	.05	.21	.15	.67	.15		.10
31	.35	.07		.06											2.31
Total	3.88	3.58	4.85	10.18	5.99	0.31	1.20	1.40	0.91	0.31	4.77	2.64	4.67	6.66	8.11
Average (10 years)	4.93	5.60	6.61	5.69	4.09	2.91	2.19	2.81	2.43	4.93	2.91	2.81	2.43	4.93	5.60

the intermediately irrigated and nonirrigated plots. Tables 7, 8, and 9 show the consumptive use of water in 123 days under experiment No. 3. It was 27.91 inches in the frequently irrigated plots, 22.70 in the intermediately irrigated, and 18.24 in the nonirrigated plots. Table 10 shows the variation in water used according to the corn growth stage. An average of 0.144 inch daily was consumed in the first 30 days after planting; this increased to an

average of 0.208 inch per day at 56 days from planting, but decreased to 0.114 inch per day during the last 41 days of the growing season. The average total consumptive use in 125 days was 18.48 inches.

TABLE 4.—*Consumptive use of water by field corn in the frequently irrigated plots of experiment No. 2, by periods, March 19 to July 21, 1957*

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*In.*	*In.*
Mar. 19 to Mar. 24[1]	6	0.492	0.082
Mar 25 to Apr. 3	10	1.052	.105
Apr. 4 to Apr. 7[1]	4	.504	.126
Apr. 8 to Apr. 17	10	1.465	.146
Apr. 18 to Apr. 21[1]	4	.880	.220
Apr. 22 to Apr. 24	3	.756	.252
Apr. 25 to May 1[1]	7	1.456	.208
May 2 to May 8	7	1.035	.148
May 9 to May 12[1]	4	.604	.151
May 13 to May 15	3	.459	.153
May 16 to May 19[1]	4	.740	.185
May 20 to May 26	7	1.625	.232
May 27 to May 29[1]	3	.792	.264
May 30 to June 2	4	1.152	.288
June 3 to June 5[1]	3	.780	.260
June 6 to June 12	7	1.526	.218
June 13 to June 16[1]	4	.896	.224
June 17 to June 19	3	.688	.229
June 20 to June 23[1]	4	.808	.202
June 24 to July 4	11	1.328	.121
July 5 to July 10	6	.468	.078
July 11 to July 21	11	.486	.044
Total	125	19.992	

[1] Consumptive-use data calculated by interpolation.

EFFECTS OF IRRIGATION

In experiments Nos. 1 and 3 there was no statistically significant difference in production due to irrigation (table 11). In experiment No. 2 there was a highly significant irrigation effect on production (table 11 and figure 1). As shown in table 11, there was no statistical difference in production between the frequently and the intermediately irrigated plots. However, there was a highly significant difference in production between the irrigated (both frequently and intermediately) and the nonirrigated plots. There was an average increase in production due to irrigation of 25.03, 34.48, and 36.01

TABLE 5.—*Consumptive use of water by field corn in the intermediately irrigated plots of experiment No. 2, by periods, March 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*In.*	*In.*
Mar. 19 to Mar. 24[2]	6	0.492	0.082
Mar. 25 to Apr. 3	10	1.052	.105
Apr. 4 to Apr. 7[2]	4	.504	.126
Apr. 8 to Apr. 17	10	1.465	.146
Apr. 18 to Apr. 21[2]	4	.564	.141
Apr. 22 to May 1	10	1.354	.135
May 2 to May 8	7	.951	.136
May 9 to May 12[2]	4	.772	.193
May 13 to May 19	7	1.734	.248
May 20 to May 26	7	1.412	.202
May 27 to May 29[2]	3	.666	.222
May 30 to June 5	7	1.677	.240
June 6 to June 9	4	.839	.210
June 10 to June 12[2]	3	.609	.203
June 13 to June 19	7	1.348	.192
June 20 to June 26	7	1.265	.181
June 27 to July 4	8	.767	.096
July 5 to July 10	6	.456	.076
July 11 to July 21	11	.583	.053
Total	125	18.510	

[1] Consumptive-use data from Mar. 19 to Apr. 17 are the same as for the frequently irrigated plots. All soil-moisture samples taken under each moisture treatment were combined to calculate the moisture extracted during that period.

[2] Consumptive-use data calculated by interpolation.

TABLE 6.—*Consumptive use of water by field corn in the nonirrigated plots of experiment No. 2, by periods, March 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use.	Average daily consumptive use
	Days	*In.*	*In.*
Mar. 19 to Mar. 24[2]	6	0.492	0.082
Mar. 25 to Apr. 3	10	1.052	.105
Apr. 4 to Apr. 17	14	1.705	.122
Apr. 18 to May 1	14	1.245	.089
May 2 to May 15	14	1.576	.112
May 16 to May 26	11	.512	.046
May 27 to June 9	14	.337	.024
June 10 to June 26	17	1.758	.103
June 27 to July 21	25	.236	.010
Total	125	8.913	

[1] Consumptive use from March 19 to April 3 is the same as for the frequently and intermediately irrigated plots. All soil-moisture samples taken under each moisture treatment were combined to calculate the moisture extracted during that period.

[2] Consumptive-use data calculated by extrapolation.

hundredweights per acre under the 0, 80, and 160 pounds of nitrogen applied per acre, respectively, (derived from averages of data in table 11).

EFFECTS OF NITROGEN FERTILIZATION

In all the experiments conducted nitrogen had a highly significant effect on yield at all irrigation levels, with the exception of the nonirrigated plots

TABLE 7.—*Consumptive use of water by field corn in the frequently irrigated plots of experiment No. 3, by periods, April 17 to August 17, 1958*

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*In.*	*In.*
Apr. 17 to Apr. 24[1]	8	1.424	0.178
Apr. 25 to May 1	7	1.452	.207
May 2 to May 13	12	2.077	.173
May 14 to May 18[1]	5	1.230	.246
May 19 to May 25	7	2.083	.298
May 26 to May 27[1]	2	.640	.320
May 28 to June 5	9	3.156	.351
June 6 to June 12	7	1.520	.217
June 13 to June 19	7	1.575	.225
June 20 to June 22[1]	3	.888	.296
June 23 to July 1	9	3.376	.375
July 2 to July 14	13	2.075	.160
July 15 to July 18[1]	4	.904	.226
July 19 to July 22	4	1.026	.256
July 23 to July 28	6	1.278	.213
July 29 to Aug. 3	6	.918	.153
Aug. 4 to Aug. 10	7	1.120	.160
Aug. 11 to Aug. 17	7	1.170	.167
Total	123	27.912	

[1] Consumptive-use data calculated by interpolation.

of experiment No. 2. However, the difference in production between the plots receiving 80 and 160 pounds of nitrogen per acre under all irrigation treatments was not statistically significant (table 11 and figure 2). In other words, the effect of nitrogen on yield follows the law of diminishing returns (figure 3). There was an average increase in production of 11.95, 6.20, and 10.47 hundredweights per acre with the application of 80 pounds of nitrogen per acre in experiments Nos. 1, 2, and 3 (derived from averages of data in table 11). As shown in table 11, the increase in production under frequent irrigation with 80 pounds of nitrogen applied per acre in experiment No. 2

TABLE 8.—*Consumptive use of water by field corn in the intermediately irrigated plots of experiment No. 3, by periods, April 17 to August 17, 1958*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*In.*	*In.*
Apr. 17 to Apr. 24[2]	8	1.424	0.178
Apr. 25 to May 1	7	1.452	.207
May 2 to May 13	12	2.077	.173
May 14 to May 20[2]	7	1.526	.218
May 21 to June 1	12	3.140	.262
June 2 to June 5	4	.942	.236
June 6 to June 12	7	1.902	.272
June 13 to June 19	7	2.013	.288
June 20 to July 1	12	4.032	.336
July 2 to July 14	13	1.075	.083
July 15 to July 28	14	1.006	.072
July 29 to Aug. 3	6	.437	.073
Aug. 4 to Aug. 10	7	.721	.103
Aug. 11 to Aug. 17	7	.954	.136
Total	123	22.701	

[1] Consumptive-use data from April 17 to May 13 were calculated from those representing a combination of all the soil-moisture samples taken under the 3 moisture levels.

[2] Consumptive-use data calculated by interpolation.

TABLE 9.—*Consumptive use of water by field corn in the nonirrigated plots of experiment No. 3, by periods, April 17 to August 17, 1958*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*In.*	*In.*
Apr. 17 to Apr. 24[2]	8	1.424	0.178
Apr. 25 to May 1	7	1.452	.207
May 2 to May 13	12	2.077	.173
May 14 to June 1	19	1.376	.072
June 2 to June 5	4	1.233	.308
June 6 to June 12	7	1.222	.174
June 13 to June 19	7	1.184	.169
June 20 to July 1	12	3.267	.272
July 2 to July 14	13	1.268	.098
July 15 to July 28	14	1.187	.085
July 29 to Aug. 3	6	.733	.122
Aug. 4 to Aug. 10	7	.868	.124
Aug. 11 to Aug. 17	7	.944	.135
Total	123	18.235	

[1] Consumptive-use data from April 17 to May 13 were calculated from those representing a combination of all the soil-moisture samples taken under the 3 moisture levels.

[2] Consumptive-use data calculated by extrapolation.

TABLE 10.—*Average consumptive use of water (inches) by field corn at different growth stages in the intermediately irrigated and nonirrigated plots of experiments Nos. 2 and 3*

Growth stage (days after planting)	Average daily consumptive use for—		Average daily consumptive use for Nos. 2 and 3	Total consumptive use for period of growth
	No. 2	No. 3		
0 to 30	0.117	0.172	0.144	4.320
31 to 55	.146	.130	.138	3.450
56 to 84	.225	.192	.208	6.032
85 to 125	.118	.109	.114	4.674
Total	—	—	—	18.476

TABLE 11.—*Effect of irrigation and nitrogen fertilizer on the yield per acre (hundred weights) of 15-percent-moisture shelled corn grown during 3 experiments in Santa Isabel clay at Lajas, P. R., 1956–58*

Irrigation treatment[1]	Effects of indicated pounds of nitrogen applied per acre in experiment—									Mean
	No. 1			No. 2			No. 3			
	0	80	160	0	80	160	0	80	160	
Frequent	22.91	37.38	37.06	29.72	40.97	42.40	20.54	33.97	39.06	33.78
Intermediate	25.54	37.02	38.45	31.14	38.60	41.05	21.07	33.46	35.08	33.49
No irrigation	26.52	36.43	37.23	5.40	5.30	5.71	22.10	27.69	29.46	21.76
Mean	24.99	36.94	37.58	22.09	28.29	29.72	21.24	31.71	34.53	29.68

[1] 1 irrigation applied to the plots with the nonirrigation treatment in experiments Nos. 1 and 2 to establish the crop.

FIG. 1.—Response of corn to irrigation when grown at a rate of 19,400 plants per acre and fertilized at a rate of 160 pounds of nitrogen per acre in experiment No. 2: 133, nonirrigated; 233, intermediate irrigation; 333, frequent irrigation.

was of 11.25 hundredweights per acre. It can be also observed in table 11 that the production in the plots receiving no nitrogen was larger when they were not irrigated, except in experiment No. 2 in which the nonirrigated plots did not receive enough rainfall for normal crop growth.

FIG. 2.—Response of corn to nitrogen when grown at a rate of 19,400 plants per acre under frequent irrigation in experiment No. 2: 313, no nitrogen applied; 323, 80 pounds of nitrogen applied per acre; 333, 160 pounds of nitrogen applied per acre.

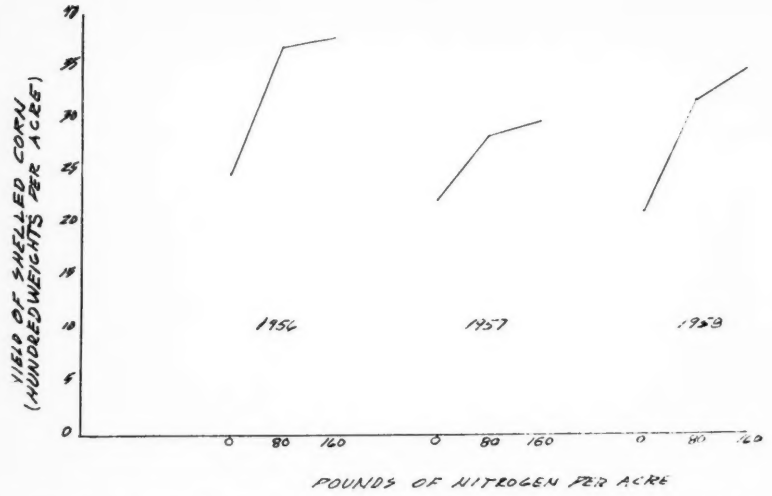

FIG. 3.—Effects of 3 different levels of nitrogen on yields of corn grown under irrigation and nonirrigated in Santa Isabel clay at Lajas, P. R., 1956–58.

EFFECT OF PLANT POPULATION

In the first two experiments conducted there was a highly significant effect of plant population on yields at all irrigation levels, except in the nonirrigated plots in experiment No. 2 (table 12). As shown in table 12, in experiment No. 2 there was an average increase in production of 5.86 and 12.07 hundredweights per acre with an increase in population from

TABLE 12.—*Effect of irrigation and plant population on the yield per acre (hundredweights) of 15-percent-moisture shelled corn grown during 3 experiments in Santa Isabel clay at Lajas, P. R., 1956–58*

Irrigation treatment[1]	Effects of indicated number of plants per acre in experiment—									Mean
	No. 1			No. 2			No. 3			
	9,600	14,500	19,400	9,600	14,500	19,400	14,500	19,400	24,900	
Frequent	28.05	31.59	37.71	32.92	37.48	42.67	30.09	30.52	32.96	33.78
Intermediate	26.76	34.39	37.86	30.77	39.20	40.82	28.75	32.20	30.70	33.49
No irrigation	26.10	32.51	41.56	6.91	5.30	4.20	26.48	26.54	26.23	21.76
Mean	26.97	32.83	39.04	23.53	27.33	29.23	28.44	29.75	29.96	29.68

[1] 1 irrigation applied to the plots with the nonirrigation treatment in experiments Nos. 1 and 2 to establish the crop.

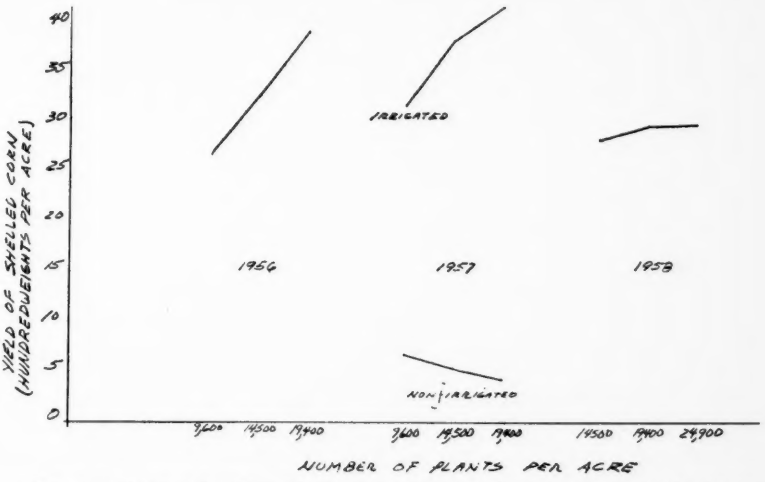

FIG. 4.—Effects of 3 different levels of plant population per acre on yields of corn grown under irrigation and nonirrigated in Santa Isabel clay at Lajas, P. R., 1956–58.

9,600 to 14,500 and from 9,600 to 19,400 plants per acre, respectively. In experiment No. 2, in the irrigated plots, the corresponding average increase in production with an increase in population from 9,600 to 14,500 plants per acre was 6.40 hundredweights per acre, and with an increase from 9,600 to 19,400 plants, was 9.80 hundredweights per acre. On the other hand, in the nonirrigated plots there was a decrease in production of 1.61 and 2.71 hundredweights per acre with an increase in population from 9,600 to 14,500 and from 9,600 to 19,400 plants per acre, respectively. As shown in figure 4, there was a linear increase in yield with an increase in plant population in the irrigated plots of experiments Nos. 1 and 2; however, in the nonirrigated plots of experiment No. 2 there was a linear decrease in yield with an increase in plant population. In experiment No. 3 there was no significant effect of plant population treatments on production (table 12).

DISCUSSION

Irrigation, nitrogen application, and increases in plant population definitely influenced corn yields. However, irrigation did not, whenever there were 20 inches of well-distributed rainfall throughout the growing season. The data obtained show that when rainfall distribution is normal (table 3), there is enough rainfall from July to November for normal growth of corn. Probably an initial heavy irrigation would be necessary at planting time. Supplemental irrigation should be applied from March to June.

Because there was no significant difference in corn yields between the frequently and the intermediately irrigated plots in experiment No. 2, or between the frequently and the intermediately irrigated, and the nonirrigated plots in experiment No. 3, the consumptive-use data from the intermediately irrigated plots of experiment No. 2, and the nonirrigated plots in No. 3 were selected as representing the normal consumptive water use of growing corn.

The growth stage of the corn plant is one of the main factors that affects the consumptive use of water. The maximum consumptive use was during the tasseling to hard-dough stage, decreasing in the last month of the growing season. In table 10 a higher consumptive use can be observed during 0- to 30-day period after planting in experiment No. 3 than in experiment No. 2. This was attributable to frequent rainfall and the impossibility of weeding during that period in experiment No. 3. Other factors like climate affect the consumptive use of water. The data need further analysis to correlate the consumptive-use data with climatic factors. Fuhriman (*3*) found that the consumptive use of water by sugarcane varied according to the growth stage of the plant and the soil-moisture levels; however, he pointed out that some climatic factors like wind movement, seasonal temperature, and hours of sunshine should be taken into consideration.

The nitrogen effect on yield follows the law of diminishing returns, *i.e.*, there was a high increase in production with the application of the first 80 pounds of nitrogen per acre, while, the difference in production when 80 and 160 pounds of nitrogen were applied per acre was not statistically significant. There was a highly significant interaction between nitrogen and irrigation. The data show that yields were increased with the same irrigation treatment when nitrogen was applied. In other words, irrigation alone does not increase yields.

A linear increase in yield was observed with an increase in plant population in experiments Nos. 1 and 2. Late weeding and fertilizing in experiment No. 3 apparently affected the plant-population effects. In experiments Nos. 1 and 2 there was a highly significant interaction between irrigation and plant population; and between plant population and nitrogen. The interaction between irrigation, plant population, and nitrogen was not statistically significant. Further work is still needed, especially on plant population and different moisture levels throughout the available moisture range.

SUMMARY

Three field experiments using three irrigation, three nitrogen, and three plant-population levels were conducted at Lajas Substation in order to determine the effect of irrigation in combination with nitrogen fertilizer and different plant populations on the production of field corn. "Frequently irrigated" plots were irrigated when 20 percent of the available moisture had been depleted from the active root zone, "intermediately irrigated" plots when the depletion was 60 percent, and "nonirrigated" plots were used as a check. The nitrogen levels tested were 0, 80, and 160 pounds per acre, and the plant-population levels were 9,600, 14,500, and 19,400 plants per acre. The data indicate that:

1. Irrigation does not influence corn yields whenever there are 20 inches of well-distributed rainfall throughout the growing season.

2. There was no significant difference in production between the frequently and the intermediately irrigated plots.

3. The average total consumptive use of water by field corn in 125 days was 18.48 inches.

4. The growth stage of the corn plant is one of the main factors that affects the consumptive use of water. During the first 30 days after planting, the corn plant consumed an average of 0.144 inches per day, increasing to an average of 0.208 inches per day during the tasseling to hard-dough stage (56 to 84 days after planting), and decreasing to an average of 0.114 inch daily in the last 41 days of the growing season.

5. There was a highly significant increase in corn yields with the application of 80 pounds of nitrogen per acre at all irrigation levels. However,

the difference in production between the plots receiving 80 and 160 pounds of nitrogen per acre was not statistically significant.

6. There was a highly significant interaction between nitrogen and irrigation.

7. There was a highly significant increase in yield with an increase in plant population from 9,600 to 19,400 plants per acre in the irrigated plots of experiments Nos. 1 and 2; however, in the nonirrigated plots of experiment No. 2, there was a decrease with an increase in plant population.

8. There was highly significant interaction between irrigation and plant population; and between plant population and nitrogen.

RESUMEN

Tres experimentos de campo para estudiar el efecto de tres niveles de riego en combinación con tres niveles de nitrógeno y población de plantas en la producción de maíz fueron llevados a cabo en la Subestación de Lajas. Los resultados fueron los siguientes: Se usaron los siguientes niveles de riego: Frecuente, cuando el 20 por ciento del agua asimilable fuera usada; intermedio, cuando el 60 por ciento del agua asimilable fuera usada; y testigo parcelas sin riego. Se estudiaron tres niveles de nitrógeno, a saber: 0, 80 y 160 libras por acre. Las poblaciónes estudiadas füeron 9,600, 14,500 y 19,400 plantas por acre.

1. La aplicación de riego no influyó sobre la producción de maíz cuando la lluvia llegó a 20 pulgadas bien distribuídas durante el período de crecimiento.

2. No hubo diferencia significativa entre la producción de las parcelas regadas frecuentemente y las regadas con menos frecuencia.

3. El promedio de consumo de agua total del maíz en un período de 125 días fué 18.48 pulgadas.

4. La etapa de crecimiento de la planta del maíz es uno de los factores más importantes que afecta el consumo de agua. Durante los primeros 30 días de crecimiento la planta de maíz consumió un promedio de 0.144 pulgadas por día, aumentando a un promedio de .208 pulgadas diarias durante el período de espigamiento hasta que el grano estuvo formado y duro (de los 56 a 84 días de sembrados), disminuyendo a un promedio de 0.114 pulgados diarias en los últimos 41 días del período de crecimiento.

5. Hubo un aumento altamente significativo en la producción de maíz con la aplicación de 80 libras de nitrógeno por acre en todos los niveles de riego; sin embargo, la diferencia en producción entre las parcelas que recibièron nitrógeno a razón de 80 y 160 libras por acre no fué estadísticamente significativa.

6. Hubo una interacción altamente significativa entre nitrógeno y riego.

7. Hubo un aumento en producción altamente significativo con un au-

mento en la población de plantas de 9,400 a 19,400 plantas por acre en las parcelas bajo riego en los experimentos números 1 y 2; sin embargo, en las parcelas sin riego del experimento número 2 hubo una disminución en la producción y un aumento en la población de plantas.

8. Hubo una interacción altamente significativa entre el riego y la población de plantas; y entre la población de plantas y el nitrógeno.

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Survey for the Presence of Tobacco Mosaic, Cymbidium Mosaic, and other Viruses in Orchids

J. Enrique Pérez and Amelia Cortés-Monllor[1]

INTRODUCTION

Orchids are grown commercially in Puerto Rico. There is evidence that the plants are frequently attacked by virus diseases which render them worthless commercially. The economic losses caused by such virus diseases may be expected to increase if radical measures are not taken to prevent their spread.

In 1956, Pérez, *et al.* (*1*)[2], were able to detect the presence of tobacco-mosaic virus in a purified preparation of *Cattleya* leaves from plants showing the "flower-breaking" disease (fig. 1). The virus was detected serologically by means of the precipitin method and also by plant-inoculation tests. Similar results were obtained from a few tests with single orchid plants.

In view of this unexpected finding it was thought best to carry out a serological survey among different orchid nurseries in the Island of Puerto Rico seeking to determine the occurrence of tobacco-mosaic virus in other orchids besides *Cattleya* and *Cattleya* hybrids, and to detect possible infection by five other common viruses: Potato virus "X"; tobacco severe etch; tobacco-ringspot virus; pepper mosaic (Potato virus Y); and Cymbidium mosaic, since this last virus is known to be widespread among orchids and is apparently confined to the *Orchidaceae* (*2, 3*). It was desired also to establish, as far as possible, a correlation between positive serological and plant-inoculation tests with orchid extracts and the presence of symptoms in the leaves and/or flowers of plants from which the extracts were derived.

A total of 13 orchid nurseries was surveyed in Puerto Rico.

MATERIALS AND METHODS

SAMPLES

Orchid plants to be tested were selected in the nursery by the orchid growers themselves, samples were collected, and the material was brought to the laboratory.

Leaf-tip samples were obtained by cutting approximately a 2-inch-square portion from apparently diseased, suspected, or normal-appearing leaves. Cuttings were made with scissors previously sterilized by dipping in 95-

[1] Bacteriologist, and Research Assistant in Bacteriology, respectively, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R. Thanks are due to Dr. D. D. Jensen for supplying Cymbidium leaves infected with Cymbidium mosaic virus.

[2] Italic numbers in parentheses refer to Literature Cited, p. 149.

percent alcohol and flaming three times. This prevented spread of infection within the nursery.

Four different kinds of samples were collected: 1, Leaves showing one or more of a variety of symptoms such as chlorosis, mottling, necrotic sunken areas, and rings; 2, normal-appearing leaves of plants which had borne flowers with the color-breaking symptom; 3, normal-appearing leaves of plants with no history or evidence of disease; 4, flowers showing the color-breaking symptom and/or other abnormalities; and 5, apparently normal white flowers from plants showing leaf symptoms.

FIG. 1.—Flower from a *Cattleya* hybrid showing gross color-breaking and distortion. Flowers such as these were shown to be infected by tobacco-mosaic and Cymbidium-mosaic viruses. (Photographed by H. R. Cibes.)

Most samples came from old leaves and older plants. A few, however, were from young leaves. Before serological testing all samples were processed by chloroform treatment as described elsewhere (*1*), but in extracting young tissue it was found that the addition of dithiocarbamate and the filtration procedure could be omitted. Both chloroform-treated and untreated clarified saps were used for the serological testing of some samples.

SEROLOGICAL TESTS

The precipitin method was used for the tests. About 0.2 to 0.3 ml. of a $^1/_{20}$ dilution of each of the following antisera was placed in a 5 × 60-mm. clean test tube: OVP (orchid virus preparation) antiserum (*1*) and specific

antisera to the following viruses: Tobacco mosaic, tobacco severe etch, tobacco-ringspot virus, potato virus X, and pepper mosaic (a strain of potato virus Y) (*5*). For the sake of clearness the viruses and their antisera will be referred to as follows: Tobacco-mosaic virus (TMV); tobacco severe-etch virus (TSE); potato virus X (J_1X); pepper-mosaic virus (PV); and tobacco-ringspot virus (RS). The technique for the preparation of OVP and TMV antisera has been described earlier (*1*). Antisera for the other viruses were similarly prepared by using partly purified preparations for the inoculation of rabbits.

To each tube was then added 0.2 to 0.3 ml. of the orchid-leaf or flower-chloroform extract to be tested. The extract and serum were mixed thoroughly by agitating the contents of each tube with a separate wooden applicator and all tubes placed in a 37°C. incubator for a maximum of 3 hours. The mixtures were observed for precipitate formation at 15, 30, 60, 120, and 180 minutes after incubation. They were then placed overnight in a refrigerator and observed again after 15 minutes additional incubation at 37°C. to remove water of condensation from the outer surface of the tubes. Controls were carried out by mixing the extracts in the test with normal rabbit serum and at least one known serologically negative extract with normal rabbit serum and each of the six antisera.

INTERPRETATION OF SEROLOGICAL TESTS

The formation of a precipitate after incubation for any of the time intervals recorded above was considered evidence of the presence of virus, provided the controls remained negative. Very few instances of nonspecific precipitation occurred with the chloroform extracts even after overnight incubation in the refrigerator.

CROSS-ABSORPTION TEST

The OVP antiserum was mixed separately both with clarified sap and chloroform extract from a Cymbidium leaf infected with Cymbidium-mosaic virus (CMV). There was a strong precipitin reaction, indicating the presence of antibodies to CMV in the OVP serum. The antiserum was then absorbed with the CMV until it no longer gave a precipitin reaction with this virus. However, a positive reaction was obtained when this absorbed serum was tested with purified TMV. Conversely, when the OVP antiserum was absorbed with TMV it still gave a reaction with CMV. This proved that the OVP antiserum contained antibodies for both CMV and TMV. Thus, in the survey, when an extract gave a positive reaction with OVP antiserum, this could be interpreted as indicating that it contained either a strain of CMV or TMV or a complex of both strains. Therefore, a reaction which was positive with OVP but negative with TMV antiserum was taken to indicate that the extract contained CMV.

OVP serum was used unabsorbed in the survey and, therefore, a positive reaction with it is recorded as "OVP". When inoculation of extracts giving this reaction gave the symptoms on *Datura stramonium* L. described by Jensen and Gold (*4*) for CMV, the results were recorded as "CMV".

PLANT INOCULATION TESTS

Twenty orchid-leaf or flower-chloroform extracts which had reacted positive with the OVP or both the OVP and TMV antisera were frozen at −10°C. until a number of young vigorously growing nonorchidaceous species were available for inoculation as test plants. A few serologically positive clarified leaf saps were similarly kept. From three to five test plants were usually inoculated with the serologically positive extracts. Five negative extracts were also carried as controls.

After thawing, a sterile cotton swab was dipped into the extract and the wet swab rubbed onto leaves previously dusted with carborundum powder. Each extract was then separately inoculated on a number of plants.

The species used were: *Nicotiana tabacum* L. (Holmes' NN having the localization factor for tobacco mosaic virus) (*6*), *N. glutinosa* L., *Gomphrena globosa* L., *N. tabacum* L. var. Virginia-12, *Lycopersicon esculentum* L. var. Marglobe, and *Capsicum frutescens* L. var. Large Bell Hot. In some tests the following species were also inoculated: *Datura metel* L., *Chenopodium album* L., *N. sylvestris* Speg. and Comes, and *Phytolacca americana* L. These plants were used to detect the presence of any of the viruses tested for serologically. *Datura stramonium* L. was also included since, as reported by Jensen and Gold (*4*) and White (*9*), this species reacts to infection with Cymbidium mosaic with slow-developing local necrotic lesions which appear about 2 weeks after inoculation and are first seen on the most mature leaves. This was our criterion for detecting Cymbidium mosaic in facing the impossibility of using Cymbidiums which do not adapt well to hot tropical climates. Uninoculated plants of all species used were kept as healthy controls.

Serological tests were performed on the inoculated plants in order to confirm the observed results.

RESULTS

Orchids from a total of 13 nurseries were included in this study. In all, 267 samples were taken. Of these, 250 were leaf-tip samples only, 12 were samples of flower and a leaf-tip from the same plant, and 5 were flower samples only. Samples were distributed as follows: 205 from *Cattleya* and *Cattleya* hybrids (*Cattleya* spp., *Brassocattleya, Laeliocattleya, Brassolaeliocattleya*); 11 *Vandas;* 9 *Epidendrums;* 8 *Oncidiums;* 4 from *Brassavola nodosa;* 3 *Dendrobiums;* 2 *Cymbidiums;* 2 *Phalaneopsis;* 2 *Arandas;* 2 *Arachnis,* and one each from the following: *Renanthera, Gongora, Schombolaelia,*

Spathoglottis, *Aerides*, *Calanthe*, and *Maxillaria*. CMV and TMV were detected together in the *Cattleyas*, *Epidendrum*, and *Calanthe;* and CMV alone in the *Cattleyas*, *Cymbidium*, *Vanda*, *Oncidium*, *Phalaneopsis*, *Arachnis*, *Aerides*, and *Schombolaelia*.

In some cases, whole plants were brought to the laboratory by growers who had noticed abnormalities on the leaves and also such symptoms as flower-breaking and reduction in size or deformation of flowers. On the leaves, the majority of plants showed one or more of the following features: Chlorosis, necrotic rings or sunken spots, discoloration, and deformation. However, there were many leaves with doubtful symptoms. It should be emphasized again that a very large majority of samples were taken from old leaves of long-standing plants. There were very few cases of true leaf mosaic. One plant of *Epidendrum ciliare*, a native species, showed symptoms of very distinct systemic mosaic and it gave a very strong precipitin test with both OVP and TMV antisera.

A large number of serologically positive extracts was obtained from leaves showing the necrotic sunken spots and the irregular elongated streaks described by Jensen and Gold for Cymbidium mosaic virus in *Cattleya* (*4*). Although most of the extracts showing a positive reaction with OVP antiserum came from leaves showing these symptoms, a reaction with TMV antiserum was observed with some of them. Thus, this was the symptom pattern better correlated with positive serological reactions. In addition, serologically positive extracts came from leaves showing other symptoms such as leaf deformation and hardening, purplish spots or areas, and chlorotic spots, rings or streaks.

Results of precipitin tests with leaf samples are given in table 1. It may be seen that of a total 207 diseased or suspected leaves, 55(26.5 percent) reacted with the OVP antiserum only, while 26(12.5 percent) gave a positive reaction with both OVP and TMV antisera. Thus, a total of 39 percent were serologically positive. When a number of the extracts that reacted with OVP serum only were inoculated on *Datura stramonium* most of them indicated the presence of Cymbidium mosaic virus (CMV) (*4*, *9*) (see plant inoculations, table 2).

On the other hand, 126 samples (61 percent) were suspected of disease yet were serologically negative. Most of these plants did not show necrosis but, instead, a variety of indefinite symptoms such as faint light- and dark-green banding, "diffuse mosaic", dryness, yellowing, lengthwise folds, and some discoloration.

Likewise none of the normal-appearing leaf samples from plants with abnormal flowers gave a positive serological test.

In table 2 it may be noted that, among the 17 flower samples 8 showed the gross color-breaking symptom and, of these, 6 reacted both with the OVP and TMV antisera, 1 reacted with OVP only and 1 was negative.

Eight other flowers presented symptoms which differed from gross color-break: 3 exhibited discolored patches or some abnormality of pigmentation in the labium, 1 had thin white streaks on 3 petals, and 1 showed atrophy of flower segments. Of these 8 flowers, 6 reacted with OVP serum and 2 were negative. On the basis of this limited number of tests it seems that gross color-breaking is more consistently associated with a reaction with both TMV and OVP. On the other hand, a pattern of incomplete or mild

TABLE 1.—*Detection of virus in orchids by precipitin tests of extracts from leaf-tip samples*[1]

Condition of leaves	Number of samples	Reaction with antisera[2]							Total negative
		OVP—TMV[3]	OVP[4]	TMV	J_1X	PV	TSE	RS	
1. Abnormal leaves: (Symptoms present or suspected—flower unknown)	207	26(12.5 percent)	55(26.5 percent)	—	—	—	—	—	126(61 percent)
2. Normal leaves:									
a. Abnormal flower reported	6	—	—	—	—	—	—	—	6
b. Normal flower reported	37	—	—	—	—	—	—	—	37
Totals	250	26	55	—	—	—	—	—	169

[1] Flowers not available for test; condition of flowers reported by growers. The minus sign indicates negative reaction.

[2] Letters designate antisera to the following viruses: OVP (orchid-virus preparation); TMV (tobacco-mosaic virus); J_1X (potato virus X); PV (pepper-mosaic virus); TSE (tobacco severe-etch virus); RS (tobacco ringspot virus).

[3] Positive both with OVP and TMV antisera.

[4] Positive with OVP antiserum only.

break is more consistent with a positive reaction with OVP alone. Only one white-flowered plant was tested (*Cattleya* Joyce Hannington) (fig. 2).[3] Both a normal-appearing flower and one leaf from this plant were positive for OVP and TMV. It may be seen that results of precipitin tests with flowers and leaves of the same plant were mostly in good agreement. However, it is evident that virus was more easily detected serologically in flowers.

Table 3 shows the results of the confirmatory tests performed with 25 orchid samples, 20 of which gave a serologically positive reaction while 5

[3] *Cattleya* hybrid.

were serologically negative samples. As previously stated (p. 141) a number of plant species including Holmes' hypersensitive NN-tobacco and *Nicotiana glutinosa* were used to confirm the presence of tobacco-mosaic

TABLE 2.—*Relationship between serological reaction of extracts from orchid flowers and leaves of the same plant, and observed symptoms*

Plant No.	Flower		Leaf	
	Symptoms	Serological reaction	Symptoms	Serological reaction
117	Color-breaking	OVP[1], TMV	Chlorotic areas	OVP
130	do.	Negative	Slightly deformed	Negative
200	do.	OVP, TMV	Necrotic crateriform lesions	OVP, TMV
229	do.	OVP, TMV	Chlorotic and necrotic lesions	OVP, TMV
244	do.	OVP, TMV	No symptoms	Negative
255	do.	OVP	Doubtful	Do.
260	do.	OVP, TMV	Chlorotic and necrotic lesions	OVP, TMV
251	Color-breaking—small flower	OVP, TMV	[2]	[2]
129	White flower	OVP, TMV	White streaks on under surface	OVP, TMV
29	Single, thin white streaks on 3 petals	OVP	Chlorotic areas	OVP
201	Deformation; atrophy of flower segments	OVP	Unknown	OVP
203	Greenish spots (insect bites)	Negative	No symptoms	Negative
204	do.	OVP	do.	Do.
240	Pigment increase on one side of labium, small flower	Negative	[2]	[2]
231	Irregular, discolored patch on labium	OVP	[2]	[2]
250	Mild color-break (underside of labium)	OVP	[2]	[2]
252	Pigment increase on one side of petal	OVP	[2]	[2]

[1] Positive reaction with OVP (orchid-virus preparation antiserum); positive reaction with TMV (tobacco-mosaic virus antiserum).

[2] No sample.

virus. *Datura stramonium* was the sole indicator used to detect CMV (*4*). Samples 13 to 23 and 103 were not tested for CMV by plant inoculation. In table 3 it may be seen that from the majority of samples which reacted

with OVP and TMV antisera and were tested on plants, both CMV and TMV were recovered (samples 28, 40, 53, 55, and 245). On the other hand, TMV was recovered from 3 samples (Nos. 17, 20, and 32) which had reacted serologically with OVP but not with TMV antiserum. In all samples serologically positive for TMV, this virus was detected by both plant inoculation and serological tests of the inoculated plants or by plant inoculation alone. Finally sample 130A, a "broken" flower which was serologically negative, yielded both TMV and CMV upon plant inoculation, but the very

FIG. 2.—A, *Cattleya* Joyce Hannington; notice necrotic streaks and sunken spots on leaves. An extract from a cut leaf-tip was serologically positive with both TMV and OVP antisera. B, A normal-appearing white flower of the same plant which, however, was also serologically positive with TMV and OVP antisera.

few local lesions produced on NN-tobacco, *N. glutinosa* and *Datura stramonium*, were an indication of the low titer of the viruses present. In like manner the sap from these solanaceous plants gave a weak precipitin reaction with OVP and TMV antisera. This may explain the originally negative serological reaction of the flower. Reactions with the other antisera were all negative.

Results obtained with sample No. 68 (*Vanda* Cobber Cain) are of particular interest. This sample reacted positive with OVP and negative with TMV antiserum. Upon inoculation on *Datura stramonium* it gave the expected delayed local lesions of CMV (*4*, *9*). Although no symptoms were obtained on either Virginia 12 tobacco or *Chenopodium album*, extracts from leaves

of these two species gave a positive serological test with the OVP serum only. This suggests that multiplication of CMV may take place in these species in the absence of expressed symptoms.

TABLE 3.—*Results of confirmation of serological reaction of extracts from orchid-leaf and flower samples through virus recovery and serology of inoculated test plants*

Orchid sample No.	Source	Reaction with antiserum	Confirmatory tests	
			Symptoms	Reaction with antiserum
13	*Brassocattleya* Anna Sladden	OVP, TMV	TMV[1]	TMV
17	*Laeliocattleya* Dulzura	OVP	TMV[1]	TMV
20	*L.* Sargon	OVP	TMV[1]	TMV
21	*L.* Isabel Sanders x (?)	OVP, TMV	TMV[1]	TMV
22	*Brassolaeliocattleya* Kensington	OVP, TMV	TMV[1]	TMV
23	*B.* Nanette	OVP, TMV	TMV[1]	TMV
28	*Cattleya* Joyce Hannington	OVP, TMV	CMV, TMV	OVP, TMV
29A	*B.* Normans Bay[2]	OVP	CMV	OVP
29B	*B.* Normans Bay	OVP	CMV	OVP
30	*B.* Dorothy Fennel	OVP	CMV	OVP
32	*B.* Nigeria	OVP	CMV, TMV	OVP
40	*C.* Ardens	OVP, TMV	CMV, TMV	OVP, TMV
53	*Epidendrum radicans*	OVP, TMV	CMV, TMV	OVP, TMV[3]
55	*E. radicans*	OVP, TMV	CMV, TMV	OVP, TMV[3]
68	*Vanda* Cobber Cain	OVP	CMV	OVP
69	*V. teres*	OVP	CMV	OVP
103	*E. ciliare*	OVP, TMV	TMV[1]	Not done
124	*L.* Tokyo rose	OVP	Negative	Negative
132	*B.* Normans Bay[4]	OVP	CMV	OVP
245	*C. Priscilla alba*	OVP, TMV	CMV, TMV	Not done
47	*C. gigas*	Negative	Negative	Negative
59	*L. Canhamiana alba*	do.	do.	Do.
109	*C. labiata*	do.	do.	Do.
113	*C. Gaskelliana*	do.	do.	Do.
130A	*L.* Cantab x (?)[5]	do.	CMV, TMV[6]	CMV, TMV (weak)

[1] Not tested for CMV on *Datura stramonium*.
[2] Flower sample—thin white streaks on 3 petals of lavender-colored flower.
[3] Reaction tested on *Gomphrena globosa* leaves only.
[4] This sample is another from same plant as 29A, tested more than 1 year later.
[5] Flower sample—color-breaking.
[6] Very few lesions obtained.

When a number of serologically positive orchid chloroform extracts was titrated against constant concentrations of OVP and TMV antisera, they gave much higher titers with the OVP ($^1/_{16}$ to $^1/_{32}$) than with TMV anti-

serum (½ to ¼), showing that CMV was found in much higher concentration than TMV in the extracts.

DISCUSSION

The results obtained from this serological survey further confirm the previous report on the presence of tobacco-mosaic virus (TMV) in *Cattleya* orchids and also show its presence in *Epidendrum* and *Calanthe*. It is interesting to observe that TMV was always found in association with Cymbidium mosaic virus (CMV). However, CMV was encountered alone in a larger number of orchid genera. This is in agreement with the findings by previous workers (*2*, *3*, *4*) of its predominating occurrence.

The fact that orchid flowers showing the color-breaking or variegation symptom were shown to contain both CMV and TMV, is of interest in connection with the sinuous-particle:rigid-particle complex found by Murakishi (*3*) and Newton and Rosberg (*10*), in association with color-break. Murakishi stated that the virus complex found in plants showing the color-break symptom is made up of a mixture of CMV (flexuous particles) and Odontoglossum ringspot virus (ORS-rigid particles). The findings here reported strongly suggest that the color-break virus complex may be made up of CMV and TMV. Plant inoculation and serological tests with ORS indicate its relationship to TMV (*11*). However, because of discrepancy in the reported size and morphology of ORS and TMV particles (*12*), a further re-examination of this problem is necessary.

Perhaps another interesting result from this survey is the finding that orchid flowers showing mild pigment disturbances were serologically positive only with OVP serum. This could signify: 1, That CMV alone is able to cause some form of pigment disturbance, or 2, that in this condition TMV is present in serologically undetectable amounts. This second explanation is the more plausible since some samples that reacted originally only with OVP serum were later shown to contain TMV by inoculation of non-orchidaceous hosts and serological testing of these hosts.

Further on, if flower color-break is caused by a complex of CMV and TMV, as our findings suggest, then the problem of insect transmission of color-break should be re-investigated. It has been shown by Jensen (*8*) that aphids may transmit color-break from flowers showing this symptom to healthy buds. If confirmed, this could mean that a virus complex involving TMV as one of its components is transmitted by aphids. This should prove a very interesting finding.

The results also show that the only viruses isolated from orchids were CMV and TMV, although tests were carried out to detect other viruses both serologically and by plant inoculation. However, the possibility of the existence of viruses which are not transmissible outside the *Orchidaceae*,

and are not serologically related to the ones included in this study, must be clearly borne in mind. In addition, the limitations of the precipitin test employed as a serological technique are clearly shown by results with samples which were serologically negative, yet were shown to contain TMV and/or CMV by plant inoculations. The results with sample 130A, already mentioned, are one example. Likewise, the negative precipitin tests obtained with the normal leaf samples from plants which had borne variegated or "broken" flowers, may be another example of the limitations of the precipitin test. These samples may have come from old infections and therefore virus may have been present on the leaves in amounts insufficient for serological detection. In like manner if these infections had been recently acquired through the flower (*8*) virus might not have yet reached serologically detectable amounts in the leaves. Pérez, *et al.* (*1*), found that after concentration and purification of virus from symptomless leaves of *Cattleyas* with the flower-breaking disease the preparation obtained had a titer of only $\frac{1}{40}$ when measured by the precipitin-endpoint method. Similarly Zaitlin *et al.* (*7*) found low yields of virus from *Cattleya* leaves.

SUMMARY

1. A serological survey carried out in 13 orchid nurseries in Puerto Rico revealed the presence of tobacco mosaic in association with Cymbidium mosaic in variegated Cattleya flowers and in leaves of *Cattleya, Epidendrum,* and *Calanthe.*

2. Cattleya flowers showing mild pigment disturbances were shown to contain CMV only in serologically detectable amounts. Most flowers displaying gross color-breaking were shown to contain in addition, TMV.

3. No definite symptom pattern could be associated with a positive serological reaction, but a large majority of samples displaying the necrotic symptoms of Cymbidium mosaic in *Cattleya* were serologically positive. The nonreliability of visual observations for virus diagnosis in orchid leaves is further stressed.

4. The limitations of the precipitin test in relation to the present study are discussed.

RESUMEN

1. Un catastro serológico, llevado a cabo con muestras procedentes de 13 orquidiarios en Puerto Rico, reveló una infección mixta compuesta por el virus del mosaico del tabaco y el virus del mosaico del Cymbidium en flores de Cattleya con jaspeado del pigmento (*color-breaking*). Se encontró la infección mixta también en hojas de los géneros *Cattleya, Epidendrum* y *Calanthe.*

2. En las flores de orquídeas que mostraban un jaspeado benigno (*mild*

breaking) se pudo identificar solamente el mosaico del Cymbidium por el método de precipitinas empleado. En las flores con jaspeado pronunciado (*gross color-break*) se encontró la infección mixta mencionada arriba.

3. No fué posible asociar las reacciones positivas serológicas con síntomas definidos; por otra parte, la mayoría de las muestras tomadas de hojas con síntomas necróticos descritos para el mosaico del Cymbidium en Cattleya, fueron serológicamente positivas. Se destaca la dificultad de diagnosticar síntomas víricos en las hojas de las orquídea por mera observación visual.

4. Se puntualizan las limitaciones de la prueba de precipitina en relación con este estudio.

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Value and Cost of Farm-Mixed Rations Used in Swine-Feeding Trials at Lajas Substation

Ismael Carlo[1]

INTRODUCTION

Many farmers are at present interested in the swine industry. The main limitation to the expansion of this industry is the cost of feed concentrates. There is need for information on the cost of producing a market animal, the quantity of feed needed, and the income to be expected from the enterprise. This paper was prepared to provide information on swine feeding, suitable rations, and the ingredients that can be utilized to greater advantage in the preparation of these rations.

MATERIALS AND METHODS

Some years ago this Experiment Station started mixing its own swine feed, using imported ingredients together with others of local origin. The aim was to produce a cheap but well-balanced ration for its own herd and to offer the pig-raiser of the Island the information thus gathered.

This study covered periods from October 1953 to July 1955. The data on three consecutive feeding trials have been used for this paper. The period extended from weaning at 56 days to 154 days of age in each feeding trial. Computation of the feed used and net gain of the pigs was thus based on 98-days periods. Only gilts and barrows were considered. The animals were fed individually.

The feed given was prepared at the Station's mixing unit. The pigs were fed *ad libitum* from self-feeders provided in the individual pens. About 45 square feet of concrete floor space was allowed to each pig. Clean water was provided at all times. The animals were weighed every 14 days and the feed consumption recorded accordingly.

RESULTS

A total of 222 pigs, 154 gilts and 68 barrows were included. The average net gain in weight was found to be 147 pounds and the average amount of feed consumed to attain this gain was found to be 595 pounds. To get the

[1] Assistant Animal Husbandman, Lajas Agricultural Substation, Agricultural Experiment Station, Río Piedras, P. R. The author wishes to express his appreciation to Antonio González Chapel and to Luis Rivera Brenes, Assistant Director, and Head of the Department of Animal Husbandry, respectively, of Agricultural Experiment Station, for their assistance in the preparation of this manuscript.

TABLE 1.—*Composition (pounds) of ration I used in swine-feeding trials*

Ingredient	Quantity	Total crude protein	Total crude fat	Total crude fiber	Total NFE
Hog concentrate, 32-percent	230	73.60	5.75	14.95	69.00
Wheat bran	120	17.40	3.60	12.00	60.00
Pulverized oats	100	12.00	4.70	10.60	60.20
Cane molasses	100	—	—	—	61.90
Yellow corn No. 2	450	42.30	15.75	13.50	292.50
Total	1,000	145.30	29.80	51.05	543.60

TABLE 2.—*Comparative net returns for market hogs grown on feed costing $3.00 to $3.50 per 100 lb.*[1]

Feed cost per 100 lb.	Feed cost to produce 147 lb. of gain	Other costs (estimated to be 20 percent of total costs)	Total cost to produce 147 lb. of gain	Total selling price of the pig at—			Net income per pig at—		
				19¢/lb	20¢/lb.	21¢/lb.	19¢/lb.	20¢/lb	21¢/lb.
3.00	17.85	4.46	22.31	27.93	29.40	30.87	5.62	7.09	8.56
3.05	18.15	4.54	22.69	27.93	29.40	30.87	5.25	6.72	8.19
3.10	18.45	4.61	23.06	27.93	29.40	30.87	4.87	6.35	7.82
3.15	18.75	4.68	23.43	27.93	29.40	30.87	4.50	5.98	7.45
3.20	19.04	4.76	23.80	27.93	29.40	30.87	4.13	5.61	7.08
3.25	19.34	4.83	24.17	27.93	29.40	30.87	3.76	5.24	6.71
3.30	19.64	4.90	24.54	27.93	29.40	30.87	3.39	4.87	6.34
3.35	19.93	4.98	24.91	27.93	29.40	30.87	3.02	4.50	5.97
3.40	20.23	5.05	25.28	27.93	29.40	30.87	2.65	4.13	5.60
3.45	20.52	5.13	25.65	27.93	29.40	30.87	2.28	3.76	5.23
3.50	20.82	5.20	26.02	27.93	29.40	30.87	1.91	3.39	4.86

[1] The average of 147 lb. of gain were made by 154 gilts and 68 barrows in a period which covered 3 feeding trials from October 1953 to July 1955. To attain this gain animals consumed 595 lb. of feed and needed 404 lb. of feed per 100 lb. of gain.

TABLE 3.—*Composition (pounds) of ration 2 used in swine-feeding trials*

Ingredients	Quantity	Total crude protein	Total crude fat	Total crude fiber	Total NFE
Yellow corn No. 2	425	36.13	16.15	10.63	293.25
Soybean-oil meal	100	44.00	.50	6.00	31.00
Cane molasses	130	—	—	—	80.60
Corn-gluten feed	50	10.50	1.00	4.50	24.50
Dried brewer's grains	100	26.00	6.00	15.00	42.00
Wheat gray shorts	100	16.00	4.50	6.00	55.00
Royal palm seeds	70	4.27	5.81	15.96	30.66
Tankage	25	12.58	1.50	.63	.63
Total	1,000	149.48	35.46	58.72	557.64

total weight of the live pig the weaning weight should be thus added. The composition of ration 1 used in the trials described here is shown in table 1.

The proximate analysis of this ration is 14.5 percent of crude protein, 3 of crude fat, 5.1 of crude fiber, and 54.4 percent of nitrogen-free extract.

Table 2 gives the comparative net returns for market hogs making 147 pounds of net gain after weaning, when consuming 595 pounds of feed, and assuming that the cost of the feed is 80 percent of the total cost of production as found in this Station, and given prices of feed and hogs on the hoof.

This Station has formulated and used other rations with similar nutritional values, but cheaper to make, such as the two presented in tables 3 and 4. The proximate analysis of the ration shown in table 3 is approximately 15.4 percent of crude protein, 3.5 of crude fat, 5.9 of crude fiber,

TABLE 4.—*Composition (pounds) of ration 3 used in swine-feeding trials*

Ingredients	Quantity	Total crude protein	Total crude fat	Total crude fiber	Total NFE
Yellow corn No. 2	450	42.30	15.75	13.50	292.50
Hog concentrate, 40-percent	100	40.00	3.00	9.00	27.00
Wheat gray shorts	50	8.00	1.75	3.00	27.50
Dried brewer's grains	50	13.00	2.50	9.00	20.00
Cane molasses	125	—	—	—	77.38
Royal palm seed	70	4.27	5.81	15.96	30.66
Pulverized oats	60	7.20	1.80	7.80	36.12
Corn-gluten feed	45	9.45	.90	4.05	19.35
Soybean oil meal	50	22.00	.25	2.75	15.15
Total	1,000	146.22	31.76	65.06	545.66

and 55.8 of nitrogen-free extract. The proximate analysis of the ration shown in table 4 is 14.6 percent of crude protein, 3.2 of crude fat, 6.5 of crude fiber, and 54.6 of nitrogen-free extract.

DISCUSSION

Special attention should be given to having adequate amounts of the constituents in the ration. Some ingredients are high in protein but are also high in fiber. Growing fattening pigs should not receive more than 6 percent of crude fiber in the ration. Ingredients high in fiber tend to be laxative. Molasses, used in liberal amounts, is laxative; 10 to 13 percent in the ration is safe. All cereals are low in calcium and therefore rations in which cereals are used liberally should be supplemented with minerals. Other ingredients, like royal palm seed and rice bran, are high in fat, and should be limited in their use because they have a tendency to produce too much fat. We have used around 10 percent in the ration successfully.

All the ingredients used in these rations may be imported from Continental United States and are available almost throughout the year.

Some ingredients such as these can be obtained locally: Cane molasses, royal palm nuts, and, in the near future, soybean-oil meal will also be available. To reduce the costs ingredients should be bought by the carload directly from the mills. Farmers using over 400 bags a month are in the best position to mix on the farm. Smaller farmers have to work cooperatively with others to get the advantages of this system.

An analysis of table 2 shows that feed cost is of primary importance in the growing and fattening of swine. One way of lowering the cost of swine feeds to an economical basis is to mix them on the farm.

Based on average prices of the ingredients for the year 1956–57, the cost of ration 1 was around $3.78 per hundredweight; ration 2, $3.49; and ration 3, $3.38 per hundredweight. Costs, of course, vary according to the variations in price of the different ingredients. When some of these ingredients, like soybean meal, become available locally, costs will probably be lower. Direct shipments also reduces the costs.

SUMMARY

Data gathered at the Lajas Substation from October 1953 to July 1955, using 222 pigs in individual feeding trials, showed that feed can be mixed at the farm with economic advantage to the farmer. There are some locally produced ingredients which can be used in the rations. Among them are molasses and royal palm nuts. Probably some others could be secured in the near future. Other ingredients still could be bought from Continental United States. Small farmers can work cooperatively in order to take advantage of the system. The rations used and their approximate costs are given in this paper.

RESUMEN

Según datos obtenidos en la Subestación Experimental Agrícola de Lajas, es ventajoso mezclar alimentos para cerdos en la propia finca. El alimento así obtenido resulta más barato y, por lo tanto, el beneficio neto por cerdo es mayor. Hay algunos ingredientes que se pueden conseguir localmente como la miel de caña y la semilla de la palma real. En un futuro cercano es posible que se puedan obtener localmente otros ingredientes. Los que ahora no se consiguen en plaza podrían importarse con relativa facilidad de Estados Unidos. Los agricultores de pocos recursos podrían trabajar en forma cooperativa para beneficiarse del sistema. En este trabajo se presentan las raciones que pueden utilizarse y su costo aproximado por quintal.

Aphids (Aphidae: Homoptera) on "Cacao" in the Dominican Republic[1]

Clyde F. Smith[2]

INTRODUCTION

During June of 1955, Dr. L. F. Martorell, Head of the Department of Entomology, Agricultural Experiment Station of the University of Puerto Rico at Río Piedras, collected two species of aphids on "cacao", *Theobroma cacao* L. in the Dominican Republic. One was *Toxoptera aurantiae* (Boyer de Fonscolombe). The other, apparently undescribed, is named in honor of L. F. Martorell. I wish to thank D. Hille Ris Lambers of Bennekom, Netherlands, for his opinions concerning this species.

DESCRIPTION

Macrosiphum Martorelli n. sp.

This species (fig. 1[3]) can be recognized by its brilliant yellow-green color: antennal hairs subequal to one-half diameter of base of antennal III; tuberculate sensoria on antennal III of alate vivipara; pale cauda bearing seven hairs; and dark cornicle with a pale base.

Alate Vivipara

Color of living material brilliant yellow-green. Cleared material: Antennae dark (antennal I and II only dusky); head dusky; legs beyond proximal ½ to ⅔ of femora dark; abdomen without pigmented areas; cornicles dark beyond basal 1/10 which is pale; wings with 1st anal and cubitus of forewing with dusky border.

Measurements

Body length 2.70–2.90; width across eyes 0.53–0.56; hairs on vertex of head 0.02–0.03; hairs on antennal III, 0.025 (slightly longer than diameter of base of antennal III); antennal III, 0.71–0.85; IV, 0.77–0.87 (antennal IV equal to or slightly longer than antennal III); V, 0.71–0.80; VI, 0.20–0.22 plus (0.111 + broken); rostral IV plus V, 0.14; width 0.06–0.07; hind tibia 2.23–2.38; hind tarsi 0.12–0.13; cornicles 0.69–0.80 (0.15 reticulated); cauda, length 0.31–0.34, width 0.11.

[1] Contribution from the Entomology Department, North Carolina Agricultural Experiment Station, Raleigh, N. C. Published with the approval of the Director of Research as Paper No. 1072 of the Journal Series.

[2] Head, Department of Entomology, North Carolina State College, Raleigh, N. C.

[3] Drawings by A. T. Olive.

Antennal III with 12–14 sensoria; rostrum attaining 2nd coxae; cauda with three lateral hairs and one dorsal hair. First tarsal chaetotaxy 3–3–3. Eighth tergite with four hairs.

Apterous Vivipara

Color of living and cleared material similar to alate vivipara.

Measurements: Body length 2.40–2.65; width across eyes 0.53–0.54; hairs on vertex of head 0.03; hairs on antennal III, 0.03 (equal to ⅔ diameter of base of antennal III); antennal III, 0.87–0.95; IV, 0.75–0.84; V, 0.76–0.79; VI, 0.22 plus 1.42–1.48; rostral IV plus V, 0.13–0.14; width 0.06–0.07; hind tibia 2.40; hind tarsi 0.12; cornicles 0.80–0.85 (reticulated area 0.15); cauda, length 0.36–0.40, width 0.12–0.14.

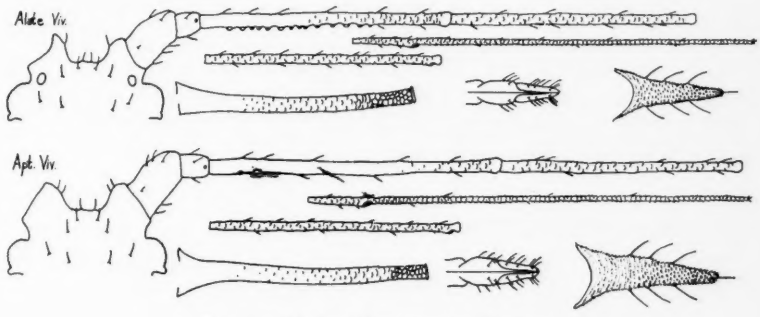

FIG. 1.—*Macrosiphum martorelli* n. sp.

Antennal III with 1–3 sensoria; rostrum attaining between 2nd and 3d coxae; cauda with three lateral hairs and one dorsal hair; 1st tarsal chaetotaxy 3–3–3. Eighth tergite with four hairs.

Types

Holotype in U. S. National Museum; paracolonotypes in U. S. National Museum, Agricultural Experiment Station of the University of Puerto Rico, Hille Ris Lamber's collection, and North Carolina State College.

Type Locality

Dominican Republic, Island of Hispaniola, W. I.

Collections

On the chocolate tree or "cacao", *Theobroma cacao* L. in the farm of Pedro Paulino, Sección La Mesa at San Francisco de Macorís, Dominican Republic, June 1955, by L. F. Martorell. Described from the holotype slide

and nine paracolonotype slides containing a total of 33 specimens. Many of the specimens are in poor condition, lacking parts of legs and antennae.

Economic Importance

This yellow-green aphid attacks the flowers, young pods and tender foliage of "cacao". The insect lives in association with the "caribe" or fire ant, *Solenopsis geminata* Fabr. On Pedro Paulino's farm Martorell observed an intense attack of this aphid, also in association with *Toxoptera aurantiae* causing serious damage, particularly to the tender growth. Wolcott (*1*)[4] in 1921 did not observe the insect attacking "cacao" in the Dominican Republic. This insect could be a potential pest of "cacao" in future years and thus it should be considered as such.

SUMMARY

A new species of aphis, *Macrosiphum martorelli* is described from the Dominican Republic. This yellow-green aphis attacks the flowers, young buds and tender foliage of "cacao", *Theobroma cacao* L. causing considerable injuries. Although of no economical importance at present it could be of some importance to the "cacao" industry in the future.

RESUMEN

Una nueva especie de pulgón, *Macrosiphum martorelli*, se describe de la República Dominicana. Este pulgón amarillo-verdoso ataca las flores, capullos y follaje tierno del cacao, *Theobroma cacao* L., y causa daños considerables. Aunque ahorta no es de importancia económica, podría ser de alguna importancia en la industria cacaotera de aquel país en el futuro.

LITERATURE CITED

1. Wolcott, G. N., Las plagas del cacao en Santo Domingo y algunas indicaciones de cómo combatirlas, *Rev. Agr. de P. R.* **6**(6) 11–2, San Juan, Junio, 30, 1921.

[4] Italic numbers in parentheses refers to Literature Cited, p. 156.

New Species of Aphidae: Homoptera from Puerto Rico[1]

Clyde F. Smith[2]

INTRODUCTION

The following new species of aphids were collected in Puerto Rico during 1954 and 1955, when the author was making a survey of the host plants of the green peach aphid, *Myzus persicae* (Sulzer) in cooperation with the Department of Entomology of the Agricultural Experiment Station, University of Puerto Rico. Unless otherwise indicated all of the material was collected by the author in cooperation with Mrs. Clyde F. Smith, Dr. L. F. Martorell, and Dr. Mario E. Pérez-Escolar.

Acyrthosiphon bidenticola Smith, n. sp.

This species (fig. 1) differs from *A. bidentis* Eastop in the unguis being much longer and the cornicles darker. *A. bidentis* also has a conspicuous median process.

Apterous Vivipara

Living specimens bright green, darker green at base of cornicles. Cleared specimens dark on antennal segments III, IV, V, and VI, cornicle (except base), legs beyond base of femora, and rostral IV + V. Dusky on antennal segments I, II, and base of III, coxae, trochanter, base of femora, base of cornicle and cauda. Remaining portions of body pale.

Length of body 1.87[3] (1.66–1.98); width of head 0.44 (0.43–0.48); hairs on vertex of head 0.01; hairs on antennal segment III, 0.01; hind tibia 1.56 (1.56–1.89); hind tarsi 0.09 (0.09–0.11).

Antennal segments III with 1 and 2 (1–5) sensoria; rostrum attaining

[1] Contribution from the Entomology Department, North Carolina Agricultural Experiment Station, Raleigh, N. C.

[2] Head, Department of Entomology, North Carolina State College, Raleigh, N. C. I wish to thank the following individuals who assisted in this study: Dr. D. Hille Ris Lambers, Dr. A. N. Tissot, Dr. W. R. Richards, and Miss Louise Russell for their opinions concerning these species; Director Arturo Roque, of the Agricultural Experiment Station of the University of Puerto Rico, for providing travel funds; Dr. G. N. Wolcott for assistance and encouragement in making this study possible; Mr. A. T. Olive for drawing the illustrations; Dr. L. F. Martorell for identification of the host plants and Dr. Martorell, Dr. M. E. Pérez-Escolar and Mrs. C. F. Smith for assistance in collecting the aphids.

[3] Measurements are in millimeters. The first number represents the measurements of the holotype, the numbers in parentheses represent the variation of the specimens which were measured.

2nd coxae; cauda with three pairs of lateral hairs, and 1 dorsal hair. Cornicles with one or two rows of faint reticulations.

Alate Vivipara

Color of living specimens bright green, darker green at base of cornicles. Cleared specimens dark on antennae, including I and II, (base of III pale), cornicles (except base), and legs (except base of femora). Slight shading along wing veins. Remaining areas pale to slightly dusky.

Length of body 1.31–2.05; width of head 0.39–0.44; hairs on vertex of head 0.02; hairs on antennal segment III, 0.01; hind tibia 1.60–1.69; hind tarsi 0.09–0.11.

Antennal segment III with 12–16 sensoria; rostrum attaining 2nd coxae; cauda with three pairs of lateral hairs and one dorsal hair. Cornicles with 2–3 rows of faint reticulations.

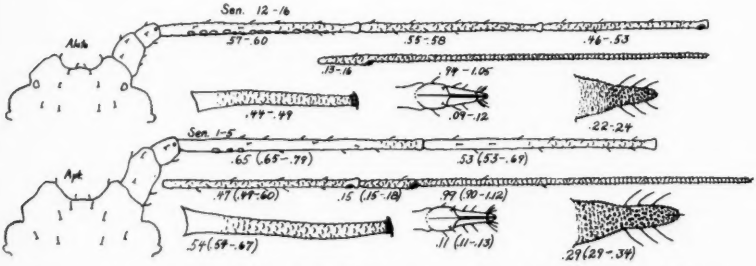

FIG. 1.—*Acyrthosiphon bidenticola* Smith, n. sp., alate and apterous vivipara.

Types

Holotype in the U. S. National Museum; paracolonotypes in the collection of the author, Agricultural Experiment Station of the University of Puerto Rico, D. Hille Ris Lambers, Netherlands. Paratype in the collection of the author.

Type Locality

Maricao Forest, P. R.

Collections

On "margarita silvestre" or "alfilerillo", *Bidens pilosa* L. (No. 55–192), Maricao Forest, Km. 15 Maricao-Sabana Grande Road, March 10, 1955, (Smith, Martorell, and Pérez), (11 slides).

On "achicoria de cabra", *Erechtites valerianaefolia* D. C. (No. 54–91), Cayey, 1,500 ft. altitude, August 2, 1954 (Smith and Pérez), (1 slide).

***Amphorophora commelinensis* Smith, n. sp.**

The chief distinguishing characteristics of this species (fig. 2) are the dark, saddlelike markings on the abdomen, the relatively small number of sensoria on antennal segment III, and the dark cornicles.

Apterous Vivipara

Living specimens whitish-yellow to yellowish. Cleared specimens dark on antennal segment VI and joints of antennae, tip of cornicle, and all of tarsi.

Length of body 1.32–1.87; width across eyes, 0.40–0.44; hairs on vertex of head 0.02; hairs on antennal segment III, 0.01; hind tibia 1.18–1.27; hind tarsi 0.09–0.10; rostrum attaining 3d coxae.

Antennal III with 0–2 sensoria; rostrum attaining 3d coxae; cauda with two pairs of lateral hairs and one pair of dorso-lateral hairs.

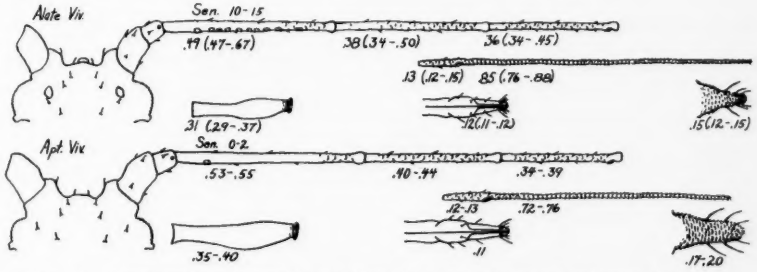

FIG. 2.—*Amphorphora commelinensis* Smith, n. sp., alate and apterous vivipara.

Alate Vivipara

Living specimens yellowish-green to light green with dark area on abdomen. Cleared specimens dark on all antennal segments (base of segments III, IV, and V may be lighter), distal ¼ of femora, distal 1/10 of tibiae, tarsi, cornicles, area partially surrounding base of cornicles which forms a dark saddlelike area across abdomen and marginal sclerites. Remaining areas of body pale.

Length of body 1.55 (1.49–1.76); width across eyes 0.40 (0.40–0.42); hairs on vertex of head 0.02; hairs on antennal segment III, 0.01; hind tibia 1.12 (1.11–1.32); hind tarsi 0.09 (0.09–0.10).

Antennal segment III with 9–10 (15) sensoria; antennal tubercles slightly convergent; rostrum attaining 3d coxae; cauda with two pairs of lateral hairs and one pair of dorso-lateral hairs.

Types

Holotype in U. S. National Museum; paracolonotypes in collection of

the author; paratypes in collections of U.S.N.M., the Agricultural Experiment Station of the University of Puerto Rico, D. Hille Ris Lambers, Netherlands, and the author.

Type Locality

Ciales-Villalba Road, Km. 32, Puerto Rico.

Collections

On "cohitre", *Commelina difusa* Burm. f. (No. 53–59) Ciales-Villalba Road, Km. 32, March 22, 1959 (Martorell and Medina-Gaud) (9 slides); (No. 55–79) Doña Juana Forest, Villalba, March 2, 1955 (1 slide); (No. 55–124) Cayey, March 8, 1955 (2 slides); (No. 55–166) El Yunque Mts., Km. 10 Mameyes-Río Blanco Road, March 7, 1955 (3 slides); (No. 55–215) Maricao Forest, Km. 19, March 11, 1955 (7 slides); (No. 55–242) Cayey-Salinas Road, Km. 6.4, altitude 1,500 ft., March 16, 1955 (4 slides); (No. 55–514) El Yunque, 1955 (Medina-Gaud, 2 slides).

On "achicoria", *Sonchus oleraceous* L., (No. 55–243) Cayey-Salinas Road, Km. 6.4, altitude 1,500 ft., March 16, 1955 (Smith and Martorell, 2 slides).

On "wild mustard", *Brassica integrifolia* (West) O. E. Schulz, (No. 55–125) at Cayey, March 8, 1955 (Smith and Martorell, 2 slides).

***Hyalomyzus jussiaeae* Smith, n. sp.**

This species (fig. 3) differs from *H. eriobotryae* (Tissot) in averaging fewer sensoria on antennals III, IV, and V of the alate vivipara, in the sensoria being confined more to one side on III and IV, sensoria being less tuberculate in the alate, and in the unguis being distinctly longer than antennal III of the alate. *H. eriobotryae* was collected at Raleigh, N. C. on *Lycopus virginicus* L. (summer host of *eriobotryae*) and the above characters were fairly constant and typical of the cotype material of *eriobotryae* received from Dr. A. N. Tissot.

Apterous Vivipara

Color of living specimens dirty greenish-brown, reddish area around cornicles. Cleared specimens with all appendages light amber, unicolorous; body pale, with a few small intersegmental sclerites along the sides.

Body length 1.30–1.90; across eyes 0.35–0.45; hairs on vertex of head 0.01; hairs on antennal III, 0.01.

Rostrum attaining 2nd abdominal segment; cauda with 2 lateral hairs. Body rugose, cleared specimens sculptured like a "dried up mud puddle."

Alate Vivipara

Color of living specimens brownish-amber. Cleared specimens with all appendages, head and thorax brownish, (femur and tibiae slightly lighter

at base). Abdomen bearing marginal, post and antesiphuncular sclerites as well as intersegmental sclerites.

Body length 1.26–1.65; across eyes 0.38–0.41; hairs on vertex of head 0.01; hairs on antennal III, 0.01.

Hairs on head small, blunt to capitate, inconspicuous. Rostrum attaining 2nd abdominal segment; cauda with two pairs of lateral hairs. Femurs rugose, with a sensorium near the base.

Types

Holotype, U. S. National Museum; paracolonotypes in collection of U.S. National Museum, North Carolina Agricultural Experiment Station, Agricultural Experiment Station of the University of Puerto Rico, D. Hille Ris Lambers, A. N. Tissot and the author. Paratypes in collections

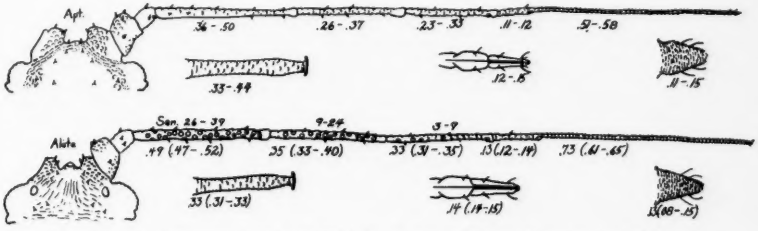

FIG. 3.—*Hyalomyzus jussiaeae* Smith, n. sp., alate and apterous vivipara.

of the Agricultural Experiment Station, University of Puerto Rico, and the author.

Type locality

Maricao Forest, Km. 219, P. R.

Collections

On "yerba de clavo" or "yerba cangá", *Jussiaea angustifolia* Lam., (No. 55-214) Maricao Forest, Km. 19, Maricao-Sabana Grande Road, March 11, 1955 (11 slides); (No. 54-47) El Yunque Mts., Mameyes-Río Blanco Road, 1 mile below swimming pool, July 26, 1954 (Smith and Pérez), (2 slides); (No. 55-509), El Yunque Mts., Mt. Britton Trail, 3000 ft. elevation, (Pérez and Martorell) (1 slide); (No. 55-163), El Yunque Mts., Mameyes-Río Blanco Road, Km. 10.5, March 9, 1955 (7 slides); (No. 73-59) Cayey-Salinas Road, Km. 11.4, altitude 1,900 ft., March 26, 1959 (7 slides); (No. 73-59) Cayey-Salinas Road, Km. 11.4, altitude 1,900 ft., March 26, 1959 (Martorell and Medina-Gaud) (5 slides).

SUMMARY

Three new species of aphids collected in different localities of Puerto Rico are described. *Acyrthosiphon bidenticola* n. sp. breeding on "alfilerillo", *Bidens pilosa* L. and "achicoria de cabra", *Erechtites valerianaefolia* D. C.; *Amphorophora commelinensis* n. sp. breeding on "cohitre", *Commelina difusa* Burm. f., on "achicoria", *Sonchus oleraceous* L., and on "wild mustard", *Brassica integrifolia* (West) O. E. Schulz; *Hyalomyzus jussiaeae* n. sp. breeding on "yerba de clavo", *Jussiaea angustifolia* Lam. Most of these species are present in middle altitudes of Puerto Rico from 1,000 up to 3,000 ft.

RESUMEN

Se describen tres nuevas especies de pulgones colectados en diferentes localidades de Puerto Rico. *Acyrthosiphon bidenticola* sp. n. se alimenta de alfilerillo, *Bidens pilosa* L. y de la achicoria de cabra, *Erechtites valerianaefolia* D. C.; *Amphorophora commelinensis* sp. n. se alimenta de cohitre, *Commelina difusa* Burm. f., de achicoria, *Sonchus oleraceous* L. y de la mostaza silvestre, *Brassica integrifolia* (West) O. E. Schulz; *Hyalomyzus jussiaeae* sp. n. se alimenta de la yerba de clavo, *Jussiaea angustifolia* Lam. Casi todas estas especies viven en las alturas medianas de Puerto Rico, entre los 1,000 y 3,000 pies de altura.

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(*Continued from inside front cover*)

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[1] As of January 1, 1960.
[2] On leave to pursue studies.
[3] On other types of leave.
[4] U.S. Department of Agriculture collaborators.